# SUPPRESSION OF *BOTRYTIS CINEREA* BY ANTAGONISTS IN LIVING, MORIBUND AND DEAD GRAPEVINE TISSUE

ANETTE VOLKMANN



**Thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Agriculture at the University of Stellenbosch**

**Supervisor: Prof. G. Holz**
**December 2001**

# DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

# SUMMARY

## SUPPRESSION OF *BOTRYTIS CINEREA* BY ANTAGONISTS IN LIVING, MORIBUND AND DEAD GRAPEVINE TISSUE

Several attempts have been made to reduce *Botrytis cinerea* grey mould in vineyards and in storage by means of biological control. However, the so called "silver bullet" approach in utilising a single antagonist, has its limitations when compared with synthetic fungicides. Often the antagonist has a limited spectrum of activity and the duration of its effectiveness is less than that provided by synthetic fungicides. Furthermore, antagonists are more likely to be effective in preventing initial infection rather than resumption of latent infection. Therefore, due to the various infection sites in grape bunches utilised by *B. cinerea* and the fact that the pathogen can remain latent in the grapevine tissue, it may be possible to obtain effective control of the pathogen by integrating fungicides and different biological control agents each aimed at a different site in grape bunches, protecting the bunch at the various phenological stages of growth and under different microclimatic conditions. In this study the potential of three fungal antagonists (*Gliocladium roseum, Ulocladium atrum* and *Trichoderma harzianum*) and one yeast (*Trichosporon pullulans*) to colonise different sites in grape bunches, and to reduce *B. cinerea* infection, was investigated in commercial vineyards. As the biological control agents were used in an integrated system, the effect of various fungicides frequently applied to local vineyards on the organisms was also investigated.

Fungicide trials were conducted taking into account two possible scenarios. Firstly, the possible effect of fungicides applied to the vineyard after an application of the biological control agent or shortly before the application of the biocontrol agent. This entailed exposing the biocontrol agents to relatively low concentrations of the active ingredient of the fungicides, similar to the residue levels to which these organisms would be exposed under field conditions. Secondly, the possibility of applying the organisms and the fungicides at the same time by making use of spray tank mixtures. This meant exposing the biocontrol agents to relatively high doses of the active ingredient of the various fungicides. Mycelial growth and germination tests were performed on agar in Petri dishes to determine the effect of fungicides. It was assumed that if the fungicide effectively inhibits the antagonist at 2.5 µg

a.i./ml, the fungicide and antagonist can not be used in an integrated programme. Based on this criterium, *T. harzianum* can not be applied to vineyards with penconazole, mancozeb/metalaxyl, pyrifenox or mancozeb. In addition *T. harzianum* can not be applied as tank mixtures with iprodione. However, *T. harzianum* can be used in conjunction with pyrimethanil, folpan, iprodione, fosetyl-Al and copperhydroxide, provided the chemicals and the antagonist are applied alternately. *Gliocladium roseum* can not be applied in a tank mixture with pyrimethanil and penconazole, but can be used on grapevine in conjunction with penconazole, pyrifenox, pyrimethanil, iprodione and fosetyl-Al. *Ulocladium atrum* can not be applied with pyrimethanil and iprodione. *Ulocladium atrum* can be applied in conjunction with penconazole, pyrifenox, pyrimethanil, iprodione, fosetyl-Al and mancozeb. The fungus can be applied in a tank mixture with penconazole and pyrifenox.

The antagonists were applied as conidial suspensions to bunches at various phenological stages in commercial vineyards planted with the wine grape cultivar Chardonnay in the Stellenbosch region, or the table grape cultivar Dauphine planted in Paarl region. Bunches were collected 2 wk after application, surface-sterilised and used for determining antagonist colonisation and *B. cinerea* infection at specific sites in the bunches. In Chardonnay, the antagonists colonised the different sites, but colonisation during the three seasons was inconsistent and sporadic. *Ulocladium atrum* and *G. roseum* colonised floral debris to a degree in the 1996 season. However, in the 1997 season these two antagonists did not develop from floral debris. *Trichoderma harzianum* colonised floral debris extensively in the 1996 season. In the 1997 season colonisation by *T. harzianum* dropped, but unlike *G. roseum* and *U. atrum*, *T. harzianum* occurred at a low level in flowers. *Ulocladium atrum* only colonised bunches during bloom, and was not found in bunches monitored from pea-size stage to véraison. This finding suggests that the saprophyte colonised moribund and dead flower parts occurring in bunches during full bloom to the pre-pea size stage, and is not likely to be found in living tissue. *Gliocladium roseum* colonised grape berries and pedicels to some degree and *T. harzianum* colonised these grape parts extensively. *Botrytis cinerea* occurred inconsistently and at low frequencies in the different sites in bunches. It was therefore not possible to comment on the effectivity of the various antagonists in the three seasons during which the trials were performed. However, it was noted that, during the pea-size stage in 1996, when high levels of *B. cinerea* were recorded, *T. harzianum* controlled these infections in the pedicels more effectively than any other treatment.

# OPSOMMING


## ONDERDRUKKING VAN *BOTRYTIS CINEREA* DEUR ANTAGONISTE IN LEWENDE, AFSTERWENDE EN DOOIE WINGERDWEEFSEL

Die benadering om *Botrytis cinerea* verrotting van wingerd met behulp van 'n enkele biologiese beheeragent in plaas van met sintetiese fungisiede te beheer, het sekere beperkinge. Antagoniste het dikwels 'n beperkte spektrum van aktiwiteit, en die duur van hul effektiwiteit is minder as dié van fungisiede. Antagoniste is gewoonlik ook minder effektief in die beheer van latente infeksie. Die patogeen het verder die opsie om druiwetrosse deur verskillende infeksieweë te koloniseer. Fungisiede kan druiwetrosse beter teen infeksie deur veelvuldige infeksieweë beskerm as 'n enkele antagonis. In die lig hiervan is die beheer van die patogeen deur 'n kombinasie van fungisiede en verskillende biologiese beheeragente, wat elk gemik is om 'n ander infeksiepunt in die druiwe te beskerm, ondersoek. Drie swamagtige antagoniste (*Gliocladium roseum, Ulocladium atrum* en *Trichoderma harzianum*) en een gis (*Trichosporon pullulans*) is in die ondersoek gebruik.

Voorloper ondersoeke, waar twee moontlike scenarios in ag geneem is, is met fungisiede uitgevoer. In die eerste scenario is die effek van fungisiede, aangewend op wingerd kort vóór aanwending van die biologiese beheeragent, of kort ná aanwending, ondersoek. Hierdie proef het die blootstelling van die biologiese beheeragent aan relatief lae konsentrasies van die aktiewe bestanddeel van die fungisied, vergelykbaar met residuvlakke waaraan die organismes onder veldtoestande blootgestel sou word, behels. Tweedens is die moontlikheid om antagoniste en fungisiede gelyktydig as spuitpompmengsels toe te dien, ondersoek. In hierdie proef is die biologiese beheeragente aan relatief hoë dosisse van die aktiewe bestanddeel van verskillende fungisiede blootgestel. Miseliumgroei en ontkiemingstoetse is op agar in Petribakkies uitgevoer om die effek van die fungisiede te bepaal. As kriterium is aanvaar dat indien 'n fungisied die antagonis effektief by 2.5µg/ml aktiewe bestanddeel inhibeer, die fungisied en antagonis nie in 'n geïntegreerde program gebruik kan word nie. Gebaseer op hierdie kriterium kan *T. harnzianum* nie aangewend word in 'n wingerd wat met penconazole, mancozeb/metalaxyl, pyrifenox of mancozeb behandel is nie. Ook kan *T. harzianum* nie in 'n spuitpompmengsel met iprodione aangewend word nie. *Trichoderma*

*harzianum* kan egter saam met pyrimethanil, folpan, iprodione en fosetyl-Al gebruik word, mits dié chemikalieë en die antagonis afwisselend aangewend word. *Gliocladium roseum* kan nie in 'n spuitpompmengsel met pyrimethanil en penconazole aangewend word nie, maar kan saam met penconazole, pyrifenox, pyrimethanil, iprodione en fosetyl-Al gebruik word. *Ulocladium atrum* kan nie saam met pyrimethanil, iprodione en fosetyl-Al gebruik word nie. Die swam kan wel in 'n spuitpompmengsel met penconazole en pyrifenox aangewend word.

In verdere proewe is die antagoniste as spoorsuspensies op trosse op verskillende groeistadia in kommersiële wingerde, wat met die wyndruifkultivar Chardonnay of die tafeldruifkultivar Dauphine aangeplant is, ondersoek. Trossies is twee weke na toediening versamel, oppervlakkig gesteriliseer en gebruik om vlakke van antagoniskolonisasie en *B. cinerea* infeksie op spesifieke nisse in die trosse te bepaal. In die geval van Chardonnay het die antagoniste die verskillende nisse gekoloniseer, maar die kolonisasie was sporadies en nie konstant gedurende die drie seisoene van ondersoek nie. *Ulocladium atrum* en *G. roseum* het blomdeeltjies tot 'n beperkte mate in die 1996 seisoen gekoloniseer, maar nie in die daaropvolgende seisoen nie. Daarteenoor het *T. harzianum* blomdeeltjies ekstensief in die 1996 seisoen gekoloniseer, en in 'n beperkte mate in die daaropvolgende seisoen. *Ulocladium atrum* kon nie trosse van ertjiekorrelgrootte tot deurslaan vestig nie. Hierdie bevinding dui daarop dat die saprofiet afsterwende en dooie blomdeeltjies, wat van volblom tot ertjiekorrelstadium in die trosse voorkom, koloniseer, maar dat dit nie in lewende weefsel voorkom nie. Daarteenoor het *T. harzianum* die verskillende trosdele ekstensief gekoloniseer. *Botrytis cinerea* het gedurende die drie seisoene wisselvallig en teen lae frekwensies in die verskillende nisse in die trosse voorgekom. Dit was gevolglik nie moontlik om 'n konkrete afleiding oor die effektiwiteit van die verskillende antagoniste as biobeheeragente van *B. cinerea* te maak nie.

In die geval van Dauphine was die onderskeie organismes swak koloniseerders van blomdeeltjies. *Trichoderma harizanum* kon egter die lewende trosdele koloniseer. Kolonisasievlakke was laag en was nooit meer as 50% nie. In beide seisoene het die kolonisasievermoë van *T. harzianum* drasties ná trostoemaak gedaal. Daarteenoor het beide *G. roseum* en *U. atrum* tydens al die ontwikkelingstadia die lewende trosdele swak gekoloniseer. *Botrytis cinerea* het ook uiters sporadies en teen baie lae vlakke voorgekom.

Die bevindinge het getoon dat klimaatsomstandighede wat in tafeldruifwingerde in die Wes-Kaap heers, nie geskik is vir die vestiging van die biologiese beheeragente wat in die studie ondersoek is nie.

In Dauphine, virtually none of the floral debris yielded the organisms in both seasons. *Trichoderma harzianum* displayed some colonisation of living tissue and developed from bunches sampled from pre-pea size to bunch closure. Colonisation levels were generally low and never exeeded 50%. The organism furthermore showed preference for colonising primarily the pedicels and berries. In both seasons colonisation by *T. harzianum* dropped drastically at véraison, and the organism did not develope from any of the sites. G*liocladium roseum* and *U. atrum,* on the other hand, displayed poor colonisation and developed erratically and at low levels from the different tissues at each sampling. *Botrytis cinerea* occurred sporadically during bunch development in Dauphine bunches. It was not possible to comment on the effectivity of the various antagonists in the two seasons during which the trials were performed. This may be ascribed to the consistently low and sporadic occurrences of *B. cinerea* in the Dauphine vineyard, and climatic conditions which did not favour the antagonists. The findings therefore indicated that climatic conditions occurring in table grape vineyards in the Western Cape province are not well suited for the establishment of the isolates of the biocontrol agents tested in this study.

# ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the following:

Prof. Gustav Holz for his constant guidance, support and patience, both during the execution of the practical work, and especially during the preparation of the manuscript;

Dr. Ilse Trautman for her endless motivation and support during this project;

Dr. Paul Fourie for his friendship, advice and motivation when it was needed most, for the willingness to share his experience and to help when needed;

Dr. Strauss Ferreira for his kind support during the project;

Sonja Coertze, Lize Havinga, Franziska Moss and Fred Walters as well as all the staff at the Department of Plant Pathology at Stellenbosch Univeristy for all the help and their overtime put into this project;

The Foundation for Research and Development and the University of Stellenbosch for financial assistance;

Tyrrel Myburgh, my husband, for his support, love and encouragement during the most difficult times of this project;

My parents, without whom studying at this University would not have been possible, and for their encouragement throughout this project.

# CONTENTS

# 1. THE BIOLOGY OF *BOTRYTIS CINEREA* ON GRAPEVINE WITH EMPHASIS ON BIOLOGICAL CONTROL

## INTRODUCTION

*Botrytis cinerea* Pers. Ex. Fr. is a devastating pathogen on grapevine (*Vitis vinifera* L.) worldwide (Nair & Hill, 1992) and is associated with early-season infection (McClellan & Hewitt, 1973; Nair, 1990; Nair & Parker, 1985) and infection of mature grapes favoured by late-season rains or prolonged periods of high relative humidity (Harvey, 1955; Jarvis, 1980). The pathogen is traditionally controlled by means of the application of fungicides in the vineyard, and the addition of sulphur dioxide emitting pads to table grape boxes destined for export. Despite these control measures *B. cinerea* is still the cause of major economic loss to both table- and wine-grape farmers in South Africa. Reasons for the lack of effective control obtained by fungicide applications is mainly due to the emergence of resistance in the pathogen populations and to a lesser extent to ineffective use of the fungicides (Pommer & Lorenz, 1982; Nair & Hill, 1992; Faretra & Pollastro, 1993; Latorre *et al.*, 1994). The quantity of fungicides applied to the vineyards is far too large to be acceptable to the consumer. For this reason and due to the lack of effectivity of the fungicides, alternative control strategies have to be investigated.

## EPIDEMIOLOGY

### Infection pathway of *Botrytis cinerea* on grapevine

In order to effectively control a pathogen it is imperative to understand clearly it's path of infection on the host in question. There are a number of schools of thought concerning the infection pathway of *B. cinerea* on grapevine. Some authors believe that infection occurs via the stamen (Pezet & Pont, 1986), while others believe it occurs via the stigma and styles (McClellan & Hewitt, 1973). Whether the pathogen can infect the berries directly through the berry surface is also still under discussion. Nelson (1956) described the infection process of *B. cinerea* on an uninjured grape berry as follows. Conidia produce a germ tube (up to 150 μm) and then an appressorium. The appressorium produces an infection peg less than 1μm in

diameter and penetrates the cuticle to form intercellular subcuticular mycelium usually restricted to the outermost 5-8 cell layers.

**Conidia.** Dry conidia can cause infection when the grapes are dusted *en masse* and kept humid (Nelson, 1951; McClellan & Hewitt, 1973). Coertze and Holz (1999) showed that single conidia deposited at several sites on a grape berry surface, can infect cold-stored, highly susceptible Dauphine berries and form separate lesions. Lesions could not, however, be initiated on fresh picking ripe Dauphine berries that seem to be resistant until they are placed in cold-storage. The cuticle and a few underlying cells of the freshly harvested berries lent the resistance. A few single conidia were able to germinate and penetrate the host surface. Most of the literature notes that mature berries are susceptible to infection and this was not the case in these experiments. In the vineyard the distribution of the conidia is most likely not in masses but as a number of single conidia deposited simultaneously on various points on the berry surface. In the vineyard the berries also dry off faster than under controlled laboratory conditions.

Spotts and Holz (1996) found that conidia of *B. cinerea* adhere more strongly to a grape berry surface when applied in a water suspension or to a wet surface of a berry, than a dry berry surface inoculated with dry conidia. This implies that raindrops deposit conidia carried on their surfaces as single cells onto the berry surfaces during runoff. Primary infections by single, dry conidia should therefore play an important role in the epidemiology of the pathogen on grape berries. Coertze and Holz (1999) found that when single dry conidia were applied to picking ripe berries, the concentration of the conidia did not affect the degree of infection at low conidial concentrations, however, as the conidial concentration increased the degree of infection increased exponentially.

**Penetration sites.** Primary penetration sites for *B. cinerea* on grape berries are the stomata and microcracks in the berry skin (Stalder, 1953; Bessies, 1972; Pucheu-Plante & Mercier, 1983). Contradictory to these findings, Coertze and co-workers (1999, 2001) found no penetration through the stomata or berry cracks, but found penetration to be direct through the berry skin irrespective of germtube length, number or branching. Pezet and Pont (1986), found that during flowering mycelial development occurs within the stamen and the associated pedicel region. However, due to the host-response mechanisms initiated by the grapevine, the pathogen is contained and remains latent in only a few cells in the grape

cluster. On the other hand, McClellan and Hewitt (1973) showed that *B. cinerea* infected the grape flowers through the stigma at the stylar end of the potential berry at bloom-time. Here the fungus then remains latent until later in the season when the pathogen renews it's growth and rots the grapes.

**Carry over inoculum and sclerotia.** Nair and Nadtotchei (1987) observed sclerotia on one-year-old canes and germination of these sclerotia lead to the production of spores likely to act as one of the primary sources of infection in grape flowers during bloom. Carry over inoculum was directly related to both flower and berry infections, and increases in carry over inoculum led to an increase in flower and berry infections (Nair *et al.*, 1995). Carry over inoculum has more effect on the flower infections than on berry infections near harvest. A prediction model designed by Nair *et al.* (1995) showed that if there is a 50% incidence of *B. cinerea* monitored on grapevines carried over from the previous season, there is a predicted 29% infection of flowers in the next season, and a 22% predicted infection of the berries at harvest. If the carry over infection is only 15%, then the predicted risk of infection of flowers is only 12%. This study clearly shows that bunch rot disease can be inoculum driven and that quantitative relationships exist between inoculum levels at carry over, flowering and harvest stages of the season.

Latent infections play a part in carry over infections. Latent infections were responsible for 58% of inoculum carried over from one season to the next (Nair *et al.*, 1995). As latent infections increased, carry over infection also increased. High levels of latent infections will lead to a high risk of floral infections in the following season.

## LATENCY

Nair and Parker (1985) found that they could isolate *B. cinerea* from apparently healthy flowers, indicating the presence of latent infections. The flowers were all symptomless, however, microscopic examination of the flowers showed myclelial growth and sporulation of *B. cinerea* on stamens, style and stigma. Nair and Parker (1985) postulated that because the stamens dehisce during the shatter period, the stigmas and the styles have to be the source of infection. Under favourable conditions these infected floral parts could expose the immature and mature berries to a high risk of *B. cinerea* infection.

Pezet and Pont (1986) posed the question for what reason a pathogen would have a latent period within the host plant. According to them Verhoeff (1980) had a number of answers to this question. He postulated that perhaps the immature berries contained a substance toxic to the pathogen which disappears with time, or the immature fruit does not contain the nutritional substances required by the pathogen and with maturity these substances may accumulate in the host tissue. The pathogen may not be able to produce the enzymes necessary for disease initiation and if the pathogen can produce these enzymes, they are deactivated by the immature fruit.

Kosuge and Hewitt (1963) speculated about the possibility of early infections, near bloom, leading to establishing latent infections in the grape berry tissue that can cause rot near maturity. Jarvis (1962) and Powelson (1960) (cited in Kosuge and Hewitt, 1963) found that mycelia that were the result of the infection of strawberry flowers can stay quiescent in the berry tissue until the berry reaches a certain maturity.

Nothover (1987) found that under field conditions the infections occurring in the floral debris remained latent. However, a few infections spread as the clusters tightened and the incidence of contact between the berries increased. In addition the berries began to mature and an increase in wet weather was observed which also favours fungal colonisation of berries.

In the 1983-1984 season, Nair and Parker (1985) found bunch rot in immature, intact and unsplit berries in mid-season after a wet period (700mm rain and 19-25 °C) in Australia. They attributed this rot development to infections developing from latent infections in the floral debris. In previous seasons when the level of latent infections was lower, less rot was observed on the immature, intact berries, even though the climatic conditions were similar to those observed in the1983-1984 season. This mid-season rot can lead to the development of high levels of inoculum that lead to a high risk of infection of berries at harvest. Nair and Parker (1985) also found that *B. cinerea* infected Shiraz berries in mid-season, but these infections did not lead to infections at harvest. This lack of disease development is the result of the bunch architecture. The Shiraz grapes were in loose bunches and the berries were therefore less compressed and better aerated than Chardonnay or Traminer grapes in the same experiment.

De Kock and Holz (1991a, 1994) hypothesise that *B. cinerea* infections developing during storage in the Western Cape in South Africa are the result of infection by inoculum present in the bunches at vèraison and later, but not the result of flower infections that have been latent in the berries till conditions become favourable.

## LEAF EXUDATES

*Botrytis cinerea* is dependent on the availability of certain sugars in the phylloplane to facilitate infection (Edlich *et al.*, 1989). Kosuge and Hewitt (1963) found that glucose and fructose are essential sugars that have to be present on the host surface to facilitate germination of *B. cinerea* conidia. Other substances present in washings collected from grape berries stimulate germination and appressoria formation. Glucose, fructose, raffinose and maltose were found to be stimulatory to the infection process (Harper *et al.*, 1981). Lactose, ribose, galactose, inositol, mannitol and sorbitol were not found to be as effective in stimulating pathogen growth. Harper *et al.* (1981) postulated that the pathogen relied on the stimulatory sugars to produce a toxin and this toxin could not be produced in such high quantities if the substrate for the pathogen was one of the less stimulatory sugars. Edlich *et al.* (1989) found that the type of sugar as well as the concentration of the particular sugar determined the infective ability of *B. cinerea* on *Vicia faba.* They found glucose and other such complete sugars (such as mannose, maltose, fructose and xylose) to be stimulatory to infection and they also found that as the sugar concentration decreased in the infection medium, the ability of the pathogen to infect also decreased.

Phenol and malic acid are present in high concentrations in grape berry exudates after bloom, but only low concentrations are found in the exudates of mature berries (Padgett & Morrison, 1990). Sugar concentrations, on the other hand, are low at bloom, but increase rapidly in later stages of ripening (Padgett & Morrison, 1990). Vercesi *et al* (1997) showed that malic and tartaric acids are poor substrates for *B. cinerea* growth and at high concentrations inhibit colony formation of the pathogen. Conversely, sugars stimulate colony formation. This suggests that the concentration, as well as the nature of the berry exudates, play a role in the colonisation potential of *B. cinerea.*

Glucose and fructose are dissolved in free water on the surface of mature grape berries (Kosuge & Hewitt, 1963). These sugars stimulate the germination of conidia of *B. cinerea.*

Availability of these nutrients increases with fruit maturity. Washings from berries stimulate elongation of germ-tubes and appressorium formation. Kosuge and Hewitt (1963) postulated that these nutrients act as a source of energy for germinating conidia and can play a role in infecting berries. Other compounds, such as amino acids also become available to the fungus in the free water on the berry surface, but have no stimulatory effect on germination of conidia. Washings from both mature and immature grape berries were equally effective in stimulating *B. cinerea* conidia and germ tube formation (Kosuge & Hewitt, 1963).

From the findings observed by Kosuge and Hewitt (1963) it seems evident that the germination of the pathogen on the host surface is dependent on the nutritional status on the berry surface. However, infection within the berry tissue is dependent on the maturity of the berry. The pathogen can germinate and form germ tubes on berries from immature to mature stage. However, rot development can only occur in mature berries, either by means of latent infections becoming active (Kosuge & Hewitt, 1963) or by means of direct penetration late in the season (Nair & Hill, 1992).

Doneche (1986) found washings from the surface of ripe berries to contain molecules of low molecular weight that stimulated germination of conidia and mycelial growth of *B. cinerea*. As the grapes matured, the sugar:acid ratio of the washings was the same as the sugar:acid ratio of the grape juice itself. The reason for the increase in sugars as the berries matured was the result of an alteration in cell-wall composition of the berry skin.

## FLORAL DEBRIS

Approximately 19% of the flowers within an inflorescence do not set fruit and become trapped in the maturing grape bunches (Nair, 1990). These flowers, if infected by *B. cinerea* can be a major source of infection within maturing grape bunches. Early season sprays can prevent the infection of these flower parts and thereby prevent subsequent infection. The composition of the floral debris within the clusters differs according to cultivar and cultural practices. Northover (1987) found that the infected floral debris consisted of stamens or more commonly of stamen filaments attached to the receptacle, dead flowers, small brown aborted embryos and loose adhering calyptra. Northover (1987) found that the percentage of ripening berries with attached floral debris varied from 74% for loose-cluster Chardonnay, to 44% for Aurore, and to 1% for Seyval. The debris consisted mainly of attached stamen

filaments. Only a small proportion of the floral debris was infected by *B. cinerea* and the total percentage of infected sites were 8.36%, 2.19% and 2.26% for loose cluster Chardonnay, Seyval and Aurora, respectively. Many of the infection sites in Seyval and Aurora were associated with split berries and infection that had spread from adjacent infected berries, some of which were devoid of floral debris. Therefore, there was no evidence that the infections were the result of infections that had arisen from the dead remains of the styles. However, he also noted that infected dead floral parts did remain in the clusters and most probably served as foci for the increase in infection occurring during berry ripening. The morphology of table grape bunches allows aborted floral debris to fall out of the grape clusters as the bunches are very loose (De Kock & Holz, 1994). Colonised necrotic floral debris were found in Barlinka bunches until late pea-size, but were not found at vèraison.

McClarren and Hewitt (1973) postulated that the point of entry of the conidia is the stigma. Midseason bunch rot of grapes in the Hunter Valley in Australia was therefore attributed to the high level of infections occurring in the vineyards during flowering (Nair & Parker, 1985; Nair *et al.*, 1995). These workers argued that these infections occurred during the pre-bloom period via the stylar end of the fruit. The pathogen then remained latent until the start of fruit development when it renewed growth and rotted fruit under optimal climatic conditions. A floral infection of 50% was predicted to lead to 17% infection of berries (Nair *et al.*, 1995). However, Pezet and Pont (1986) postulated that *B. cinerea* infect the stamens and grow down into the receptacle (pulvinulus) area of the berry and the pedicel where it may remain latent untill the berry ripens. Pezet and Pont (1986) could not find the pathogen in the stigma region and neither could Northover (1987).

## HOST RESPONSE

Pezet and Pont (1986) found that the raw extracts taken from clusters are very inhibitory to *B. cinerea* if taken from clusters from flowering to the beginning of ripening. However, once the berries become more mature this inhibitory effect disappears. Pezet and Pont (1986) reported that Hill *et al.* (1981) observed a resistance to *B. cinerea* expressed by immature berries. They attributed this resistance to the presence of tannins in the immature berries. These tannins are responsible for the inhibition of the digestive enzymes produced by *B. cinerea.*

Holz and co-workers (Coertze & Holz, 1999; Coertze *et al.*, 2001; Holz *et al.*, 1997,1998; Holz, 1999) showed that grape berries of different cultivars have a high natural resistance to airborne conidia of the pathogen, by the inability of single conidia of *B. cinerea* applied to berries at different densities to initiate infection. The cuticle and a few underlying cell layers of the berries cause resistance to varying numbers of single conidia deposited dry at several sites on the berry surface. The active defence mechanisms such as lignification (Hoos & Blaich, 1988); phytoalexin production (Langcake, 1981) and suberization (Hill, 1985) play an important role in the resistance of the grape berries to *B. cinerea.* As the berries mature, these resistance mechanisms weaken (Hill *et al.*, 1981; Creasy & Coffee, 1988). Coertze *et al.* (2001) showed that this may be the case for suberin formation, but not for stilbene accumulation. They proved this by showing that the amount of suberin induced by appressoria still increased on green berries after appressorium formation peaked. Early suberization of cell walls around infection sites on green berries, but not on ripe berries, indicates that suberization may be an important component in the resistance of green berries to *B. cinerea*. Stilbenes, on the other hand, were formed on green, ripening and picking-ripe berries. Stilbene reactions occurred earlier on green berries than on ripening berries and picking-ripe berries, but the reaction always coincided with appressoria formation which also occurred earlier on green berries than on ripening and picking-ripe berries. Coertze *et al.* (2001) indicated that active defence cannot be considered the primary determinant in resistance and a higher priority should be placed on the contribution of the berry skin components in resistance to *B. cinerea.*

The mode of *B. cinerea* infection plays a role in the defence of grapevine to pathogen attack (Coertze & Holz, 1999; Coertze *et al.*, 2001; Holz *et al.*, 1997,1998; Holz, 1999). Mature grape berries were found to be resistant when single airborne conidia were dispersed at several sites on the berry surface, but susceptible when a cluster of conidia were applied per site.

## CLIMATIC CONDITIONS

The percentage of infections occurring as the result of *B. cinerea* infection on loose cluster Chardonnay grapes increased from 6% to 54% after a 30 h (12°C) wetting period when berries were at 16°Brix (Northover, 1987). Four to seven days after this wetting period slip skin symptoms appeared in the clusters. Aerial mycelium of *B. cinerea* on grapevines

developed optimally at 21°C and a relative humidity of 94% with no wind (Thomas *et al.*, 1988). Aerial mycelium did not develop on berries exposed to 69% relative humidity and wind. Wind speed was also found to have a significant effect on *B. cinerea* development, therefore manipulations of wind speed through canopy management, planting orientation and planting density can reduce disease incidence.

Nelson (1951) found free water on the berry surface or accompanying high relative humidity or both play an important role in disease development. At a relative humidity of 94% and higher, the time that the berry is wet is of no consequence (85 to 100 % infection occurred whether the wet period was 1 or 18 hours). However, as the relative humidity dropped, the duration of the wet period had to be longer to favour disease development.

Holz and co-workers (Coertze & Holz, 1999; Coertze *et al.*, 2001; Holz, 1999; Holz *et al.*, 1997,1998) found that the penetration process differed under wet or moist conditions. On moist berries, conidia formed a short, unbranched germ tube, while germ tube growth was extensive and sometimes even more than one branched germ tube originated from a single conidium under wet conditions. Under moist conditions one protoapressorium and sometimes a simple appressorium formed while under wet conditions a variety of appressoria formed. Growth on moist berries was restricted, irrespective of berry ripeness while on wet berries growth was poor on green berries but extensive on ripening and picking ripe berries. Germling dieback was more severe on green than ripening berries and more so on wet than moist berries. This phenomenon may however also point to the detrimental effect of proanthocyanidin in skins of green berries which inhibit the macerating enzymes of *B. cinerea* and can therefore inhibit penetrating germlings of the pathogen (Hill *et al.*, 1981). It is possible that substances in the grape berry exudates will not be readily available to the fungus when conidia germinate under high relative humidity on dry berry surfaces. However, the exudates are able to dissolve in a thin water film on wet berries and can then readily be absorbed by single dry conidia deposited at several sites on the berry surface. In addition to this, diffusion should ensure a constant supply of exudates to germ tubes and hyphae of individual conidia. It was therefore postulated that when high relative humidity reigned in nature, single dry conidia had an equal ability to infect both dry and wet berries in a bunch (Coertze & Holz, 1999; Coertze *et al.*, 2001; Holz, 1999; Holz *et al.*, 1997,1998).

## WOUNDING

Northover (1987) found in experiments performed on Aurora that an average of three split berries were found per cluster. These berries were either found singly in the loose regions of the bunches, but more commonly they were found in the tight clustered part of the bunches. These split berries were colonised rapidly by fungi and provided infection points from where the pathogens spread to adjacent berries. *Botrytis cinerea* was the pathogen associated with these infection sites to the highest percentage (64%). However, *Rhizopus* spp. (33%) and *Monilinia* spp. were also found to infect the wounded berries. Wild yeasts and bacteria were also found to cause sour rot of the split berries. The loose-clustered Chardonnay cultivar had no tight bunches and therefore also no split berries. Late season increase in infection in tight clustered Seyval and Aurore grapes were attributed to splitting of mature berries and their subsequent infection by *B. cinerea.* Northover (1987) suggested that the rotting of berries not associated with dead floral debris was the result of infection occurring through macroscopic ruptures or microfissures in the epidermis, or the result of infections spreading from adjacent infected berries.

There is varying information about the importance of wounds in the infection pathway. Nelson (1956) found that Tokay grapes that had been in cold storage for 10 weeks at 0°C could be directly penetrated by *B. cinerea* conidia. Nelson (1956) postulated that this direct penetration could be the result of a reduction in resistance of the berry due to cold storage and $SO_2$ treatment. The resistance of the freshly picked grapes to direct penetration and the susceptibility of cold-stored fruit substantiate this view (Coertze & Holz, 1999).

### Insect initiated wounds

Wounds that occur in grape berries may be the result of insect attack, frost, hail, sandblasting, sun or splitting (Jarvis, 1980; Savage & Sall, 1983). Larvae of the grape berry moth *Lobesia botrana* play an important role in the spread of *B. cinerea* conidia in vineyards (Fermaud & Le Menn, 1992). These larvae can carry *B. cinerea* conidia both externally or internally and the introduction of these conidia into wounds plays an important role in the initiation of rot before veraison.

Wounds caused by the insects *Epiphas postvittana*, *Lobesia botrana* and *Drosophila* play an important role in the spread and infection of *B. cinerea*. If *B. cinerea* infection is found before vèraison the point of infection is most likely through a wound caused by an insect which exposes the susceptible fleshy part of the berry to pathogen attack (Hill *et al.*, 1981). The resistance of young grape berries is situated in the berry skin and if the pathogen is able to bypass the skin and come into direct contact with the susceptible flesh of the berry, infection can occur. However, Coertze (1999) found that a combination of fresh wounds and new inoculum is needed for successful wound infection to occur.

De Kock and Holz (1991, 1994) found no relation between early infections (bloom infections) and subsequent disease development of table grape berries in the post-harvest period. The decay that developed in the post-harvest period after a period of cold storage was the result of infections arising from inoculum present in the bunches at harvest. Coertze (1999) substantiated these findings by showing that cold-stored berries are infected by new and established single conidia of *B. cinerea* via wounds. This is due to an inability of the mature, cold stored berries to heal wounds that are readily healed by berries in the vineyards by means of suberisation.

### Mechanical leaf removal initiated wounds

Gubler *et al.* (1991) found that wounds originating as a result of mechanical leaf removal did not predispose berries to infection by *B. cinerea*, because the injuries healed quickly. They also suggested that mechanical leaf removal is a viable alternative to hand leaf removal and mechanical leaf removal is more economical and faster than hand leaf removal.

## CHEMICAL CONTROL

### Fungicides

**Benzimidazoles (benomyl, carbendazim).** These systemic, single site inhibitor fungicides were introduced in the late 1970's and were found to be exceptionally effective in the control of *B. cinerea* on grapevines (Delp, 1987). Soon after the benzimidazoles were released, resistance occurred in pathogen populations. Resistance built up so quickly because of the specific action of the fungicide (being a single-site inhibitor) and due to the exclusive use of

this new chemical in certain areas because of its incredible effectivity. Resistant strains occurred in the natural population and under constant exposure to the benzimidazoles the population shifted to be dominated by resistant strains that are as fit as the sensitive strains. Due to the fitness of the resistant strains they are still present in areas where benzimidazoles have been withheld for numerous seasons. Strategies implemented to reduce benzimidazole resistance include a reduced exposure by reducing the number of sprays applied in a season as well as making use of unrelated chemistry in combination with the benzimidazoles and by applying lower doses.

**Dicarboximides (iprodione, vinclozolin and procymidone).** The dicarboximides were released just as the benzimidazole control of *B. cinerea* was showing incompetence in the 1970's (Pommer & Lorenz, 1982). However, late in the 1970's resistance also began to show in the dicarboximides and evidence was found of cross-resistance to benzimidazoles (Fourie & Holz,1998). However, the failure in protection of the grapevines by dicarboximides was not as complete as with the benzimidazoles. The proposed reasoning for this is that the dicarboximide resistant strains are not as fit as the sensitive strains and therefore the sensitive strains can dominate in a population as soon as the population is no longer exposed to the dicarboximides. It was also found that dicarboximide resistant strains were still able to undergo conidial germination, but mycelial growth was severely hampered by the fungicide.

Due to the presence of latent infections in the grape berries, the timing of the fungicides should be looked at from an epidemiological point of view (Nair, 1990). If infection occurs during full bloom and the disease only develops later, once the berries mature, then effective control of the pathogen can only be achieved by applying sprays during bloom and the treatments before harvest will be ineffective. In greenhouse trials, Nair (1990) found that treatment at full bloom (80% cap fall) with or without treatment before bunch closure gave significantly better control of the pathogen than treatment before harvest. In field trials carried out by Nair (1990) early applications of systemic fungicides gave better control than late application. The optimum time for spraying according to these results would be a single spray applied after the first leaf unfurled. Seeing as De Kock and Holz (1994) postulate that inoculum present in the bunches at vèraison or later is responsible for post-harvest decay, it would seem effective to apply a spray at the late stage of grapevine development to eradicate the pathogen.

In the Hunter Valley there has been a shift in the timing of fungicides applied to control *B. cinerea* from post-véraison sprays to bloom sprays since Nair began with studies on the effect of early season sprays (Nair, 1990). Nair (1990) found that effective control of *B. cinerea* could be obtained with making two systemic fungicide applications during bloom. However, he found that applying the two systemic fungicides during bloom and a protective (contact) fungicide in the post-veraison period could reduce the disease even more. A number of research projects performed in Switzerland reported by Pezet and Pont (1986) also showed that early season sprays applied during full bloom were effective in controlling *B. cinerea* infections.

Northover (1987) found pea-size, tight clustered Gamay berries to be susceptible to *B. cinerea* infection. This is contrary to what most other authors have found (Nair, 1990; De Kock, 1989). Northover (1987) found a spray applied at pea-size before a wetting period was effective in controlling the amount of *B. cinerea* infection. Fungicide applications between flowering and pea-size probably reduce the infection of the floral debris. Later applications protect the ripening berries and pedicels from infection by mycelial spread from infected floral debris remaining within the clusters (Bolay & Schuepp [1967] cited in Northover, 1987). As the berries increase in size the effectiveness of the fungicides decreases because the tighter clusters hinder penetration into the clusters.

Nair (1990) also found that fungicide applications applied at bud-burst and 75% flowering help to lower the levels of inoculum in the floral debris and therefore reduced the chance of infection during mid-season and harvest. By using one spray applied at the correct time (early in the season) the levels of *B. cinerea* infection compared to the conventional spray program which makes use of four sprays, can be significantly reduced.

**Reasons for the ineffective control of *B. cinerea* by fungicides**

**Fungicide Resistance.** The first report of fungicide resistance in *B. cinerea* was found in the benzimidazole group. Smith (1988) reported field resistance to benzimidazoles after a only a few years of intensive and widespread use of this fungicide. In the mid 1970's the dicarboximides were developed and were found to be very effective against *B. cinerea* (Pommer & Lorenz, 1995). However, once again resistance to an effective fungicide was found due to the extensive use of this new fungicide group.

To delay the onset of dicarboximide resistance in *B. cinerea* Northover (1987) suggests a maximum of two iprodione treatments per growing season. He also notes that the most effective time for fungicide applications would be at pea-size or from bunch closure to early ripening (4°Brix). These applications will reduce the floral infection foci that could develop if the floral debris were to be infected by *B. cinerea*.

Fourie and Holz (1998) found resistance to both dicarboximides and benzimidazoles in most table grape growing regions in South Africa. However, the frequencies of the resistance were lower than those reported from other grape growing regions in the world (Leroux & Clerjeau, 1985; Löcher *et al.*, 1987; Beever *et al.*, 1989; Northover, 1988). It was interesting to note that benzimidazole resistant strains were still occurring in vineyards in nearly all the regions despite the introduction of the dicarboximides in the late 1970's and the subsequent reduction of benzimidazole use (Aggenbach & Marais, 1978). This shows that benzimidazole resistant strains are just as fit as benzimidazole sensitive strains, since they retained their proportion in the population after benzimidazole use had ceased.

Dicarboximide resistant strains of the pathogen were not abnormally osmotically sensitive, but had a marked reduction in fitness (Fourie & Holz, 1998). This was manifested by reduced growth on PDA by the resistant strains compared to sensitive strains. Other workers (Katan, 1982; Northover, 1983; Fraile *et al.*, 1986; Latorre *et al.*, 1994) found the same reduced mycelial growth of resistant strains.

It is evident that the more fungicide applications are applied, the higher the frequency of resistance found. Fourie and Holz (1998) found high frequencies of resistant populations in the Paarl region where climatic conditions favour *B. cinerea* infections and many dicarboximide applications have to be applied in a season to control the pathogen. In the Orange River region where climatic conditions do not favour infection by *B. cinerea* fewer applications of the single-site-inhibitors fungicides are needed and the broad-spectrum fungicides such as copper oxychloride/sulphur are sufficient to control the pathogen. In addition to this low exposure of the pathogen to the dicarboximides, the generation cycles of the pathogen are also lower under the less favourable conditions. The fewer generation cycles of the pathogen expected in the Orange River are responsible for a reduction in selection pressure in favour of resistance build-up in the pathogen population.

Beever *et al.* (1989) showed that benzimidazole resistant sub-populations are more inclined to develop resistance to dicarboximides than benzimidazole sensitive sub–populations. Fourie and Holz (1998) confirmed this hypothesis and found that 64% of benzimidazole resistant isolates were resistant to dicarboximides. A further 97% of the dicarboximide resistant populations were also resistant to carbendazim.

Beever *et al.* (1991) suggest that if dicarboximide applications before pea-size are limited to one, preferably at full bloom, the resistance incidence would not increase to alarming heights. However, Fourie and Holz (1998) found an increase in the resistance frequency in vineyards in the Western Cape province early in the season. This phenomenon is thought to be the result of the optimal conditions for disease development which reign in the vineyards from pre-bloom to pea-size stage. Many reports (Gessler & Jermini, 1985; Nair & Parker, 1985; Northover, 1987; De Kock & Holz, 1994) have been made on the role that pollen and floral debris (as a food source) and infected floral debris and aborted flowers (as an infection source) play in aiding the infection process. The nutrient source can aid the less fit resistant strains to proliferate in aborted flowers and floral debris in dicarboximide treated bunches during bloom and early set. It was shown (De Kock, 1989; De Kock & Holz , 1994) that iprodione and procymidone were ineffective in eradicating the pathogen from aborted flowers and dead floral debris early in the season.

The fungicides used to control *B. cinerea* are usually single-site-inhibitors and the risk for the build-up of resistance is high. By reducing the number of sprays applied to the vineyards the risk of resistant strains developing and the risk of selecting for resistant strains, is reduced and the re-establishment of the sensitive strains is facilitated (Nair, 1990). Alternating the single-site-inhibitor fungicides with broad spectrum fungicides can also reduce the risk of selection for resistant strains. If the dicarboximides are withheld, the sensitive population is restored due to the reduced fitness of the resistant population.

Latorre *et al.* (1994) found cross-resistance among dicarboximide fungicides and dichloran and PCNB in grapevines in Chile. They found resistant isolates to be less fit (they had an higher osmotic sensitivity) but they were still virulent. In Chile the frequency of *B. cinerea* isolates that grew on agar amended with 10 mg/L vinclozolin increased from 2% to 75.5% between the 1987/1988 and the 1993/1994 growing seasons in table grape vineyards treated twice a year with dicarboximide fungicides. These were mostly low level resistant isolates

with $EC_{50}$ values below 9.02mg/L. There was no report of complete failure of *B. cinerea* infections in any of the vineyards tested. Conidial germination is not influenced by the dicarboximide as severely as mycelial growth, which was inhibited severely in low level resistant isolates. This shows that when the pathogen population is exposed to dicarboximides, the sub-populations developing resistance are able to overcome the inhibitory effect of the chemical on germination. However, mycelial growth is still inhibited by the dicarboximide and this explains the lack of total loss of effectivity of these fungicides. This phenomenon is also the reasoning behind having to perform both conidial germination as well as mycelial growth studies when determining the resistance in a pathogen population to dicarboximides.

**Plant Architecture.** Fungicides are used widely in Californian vineyards to control *B. cinerea*, but these sprays become less effective as the grapevine matures because of heavy canopy growth and bunch closure (Gubler *et al.,* 1987). By the time the third fungicide spray application is made (at or near vèraison), it is almost impossible to penetrate the canopy with enough volume of fungicide to adequately protect the clusters.

## POST-HARVEST CONTROL

### $SO_2$-emitting pads

De Kock and Holz (1994) noted that the only way to control *B. cinerea* in the post-harvest period is by placing $SO_2$-emitting pads in the grape boxes. It was not possible to achieve control of the pathogen in the post-harvest period by applying fungicides in the vineyards. They found that applying the dicarboximide iprodione in combination with sulphur during vèraison and pre-harvest periods reduced the amount of *B. cinerea* but did not eliminate rot in the stored grapes. Control of the pathogen in the stored grapes could only be achieved by the addition of $SO_2$-emitting pads in the grape boxes.

Infections occurring in the post-harvest period are most likely the result of latent infections and not of inoculum present on the berry surface as the $SO_2$ kills the surface inoculum (Harvey, 1955).

**$SO_2$-Damage.** $SO_2$–damage, in the form of bleaching on the berry surface, appears to stimulate latent infections in Waltham Cross berries (Taylor *et al.*, 1990). The infections developing after $SO_2$–damage, come from within as $SO_2$ emitted from generators after packing should have surface sterilized the berries. They found that the role of $SO_2$ as predisposing factor is cultivar-dependant. László *et al.* (1981) found that $SO_2$–damage increased with a delay in cooling of the grapes after packing

**Residues.** László *et al.* (1981) described the mechanism of action of the $SO_2$–emitting pads as follows. High relative humidity is created inside the boxes by placing the berries into polyethylene bags. The moisture reacts with the sodium metabisulphite in the generators and $SO_2$ is emitted, killing *B. cinerea* spores present on the berry surface. The $SO_2$ can also penetrate into the berries themselves, and after two hours, they found 20 ppm $SO_2$ on the berries. This $SO_2$ reacts with sugars, galacuronic acid and other compounds in the berries to form stable complexes (Amerine *et al.* [1974] cited in László *et al.*, 1981). This bound sulphur has no fungicidal activity and is therefore lost in the control against *B. cinerea*. By reducing the time that it takes to cool the fruit down after packing, the rate of sulphur release is reduced, making the pads effective for a longer period. By allowing a large surge of sulphur just after the grapes have been packed (facilitated by high temperature), the sulphur is released quickly and taken up by the grapes and subsequently there is not enough sulphur left to protect the grapes through the entire transport system.

## CULTURAL CONTROL METHODS

### Leaf removal

Leaf removal decreases disease incidence due to the changes brought about in microclimatic conditions (English *et al.*, 1989). They found wind speed to be affected most by leaf removal and found average wind speed in canopies with leaves removed to be three to four times those of unaltered canopies. Zoecklein *et al.* (1992) found leaf removal to reduce the incidence of *B. cinerea* grey mould and of sour rot, as well as a reduction of the concentration of rot organism metabolites such as glycerol, acetic acid, gluconic acid and ethanol. Percival *et al.* (1994) also found that leaf removal has the most pronounced effect on bunch rot incidence and severity. By making use of mechanical leaf removal and removing leaves from both sides of the fruiting zone of vigorous vines early in the season, the incidence of bunch

rot was reduced by 20% and the percentage of clusters with slight symptoms of bunch rot by 17%. However, they also found an increase in fruit yield resulting from leaf removal, which Zoecklein *et al.* (1992) did not find. Gubler *et al.* (1987) found leaf removal treatments applied to Chenin blanc vineyards in Monterey (California) to reduce disease incidence from 11.9% to 1.8% and from 55.0% to 23.9% in 1984 and 1985, respectively. Excellent disease control was achieved even though climatic conditions were favourable for *B. cinerea* infection. Gubler and Bettiga (unpublished data cited in Gubler, *et al.*, 1987) furthermore showed that leaf removal resulted in an increase in spray coverage within the canopy.

## BIOLOGICAL CONTROL

Due to the many different penetration sites available for the pathogen and the fact that the pathogen can remain latent in the grapevine tissue, the control of this disease is very complicated. An effective biological control agent will have to be versatile to be effective in controlling the pathogen. It is unlikely that a single antagonist will be able to control the pathogen at these different sites and therefore an array of biocontrol agents might be more effective. It may be possible to obtain effective control of the pathogen by using a number of different biological control agents each aimed at a different niche on the grapevine, protecting the plant at the various phenological stages of growth and under different microclimatic conditions as the season progresses.

O'Neill *et al.* (1996) reported on experiments investigating the efficacy of *Trichoderma harzianum* T39, from experiments conducted in 19 countries from 1988 to 1994 on 34 grapevine varieties. One hundred and thirteen experiments were conducted in total. It was reported that in general, the reduction of disease achieved by *T. harzianum* was lower than that obtained by chemical fungicides: 36.3 ±2.7% disease reduction in the biocontrol treatments and 52.3±2.6% in exclusively chemical treatments. It was also reported that, in general, the biocontrol treatments were less effective than the chemical fungicides, regardless of disease pressure. When *T. harzianum* was applied alternately with chemical applications, the mean control efficacy of these treatments was 55.8%±3.2% and when the chemicals were applied at the critical periods only (namely the same periods at which they were applied in the integrated programme) the mean control efficacy was 44.2±4.9%. The better effect of the integrated programme than that of the partial chemical treatment indicates a benefit from inclusion of the biocontrol preparation in the integrated programme. The control efficiencies

achieved by *T. harzianum* and the partial chemical treatment (applied only at the stage when chemical applied in the IPM system) were inferior to those achieved by the exclusively chemical treatment. The control efficacy achieved by the integrated treatment was, in general, very close to that achieved by the exclusively chemical programme. This means that the integrated treatment offers an opportunity to reduce the number of chemical applications, with no substantial reduction in disease control efficacy. This integrated system is advantageous because of the reduced risk of chemical residues occurring in the harvested fruits and it reduces the risk of resistance build-up in the pathogen population towards chemicals (Gullino & Garibaldi, 1982).

In trials conducted in Croatia (Topolovec-Pintaric *et al.* 1999) on Pinot Gris vineyards in Bozjakovina from 1992 to 1995, it was found that the most efficient fungicide for controlling grey mould was dietophencarb (Powmyl), followed by tebuconazole + dichlorfluanid (Folicur E ), followed by *T. harzianum* (Trichodex$^{T-39}$), followed by an alternation between *T. harzinaum* (Trichodex $^{T-39}$) and iprodione (Kidan) and lastly, iprodione (Kidan) alone. Once again the purely chemical treatment performed better that the purely biological treatment. It is important to note the poor performance of iprodione. A likely reason for the poor performance of this chemical is the fact that the pathogen may have built up resistance to this chemical. These authors found that Trichodex$^{T-39}$ had a minimal influence on fermentation and the must from grapes sprayed with Trichodex$^{T-39}$ fermented as quickly as the control. Dichlofluanid, on the other hand, greatly inhibited fermentation of must.

In field trials performed on grapevines in New York in 1990, Harman *et al.* (1996) found that the biocontrol agents (*T. harzianum* strains P1 and 1295-22 and *T. virens* strain 31) gave statistically significant control of disease, but that this control was substantially less effective than 3-5 applications of iprodione. All three biocontrol agents tested in these trials were isolated from places other than the grapevine phylloplane. *Trichoderma virens* strain 31 was isolated from soil (Smith *et al.*, 1990), *T. harzianum* strain 1295-22 was prepared by protoplast fusion between two fungi isolated from soil (Stasz *et al.*, 1988) and *T. harzianum* strain P1 was an iprodione resistant strain, isolated from wood shavings. Even though these isolates were recovered from environments very different to the environment in which they were tested to control *B. cinerea*, they could control the pathogen in this 'new' niche. Strain 1295-22 could control *Pythium ultimatum* Traw., *Rhizoctonia solani* Kühn., *Fusarium* spp., and *Sclerotium rolfsii* Sacc. (Harman *et al.*, 1996). This strain can also control soil pathogens

on a host of crops and in diverse geographical regions. Broadly effective strains are good candidates for commercialisation since they can be used for a variety of applications on diverse crops and in diverse geographical locations. Strain 1295-22 is registered with the EPA in various formulations, as microbial pesticides. Toxicity testing of this organism has revealed no detrimental effects to vertebrate test animals (TGT Inc. Geneva, NY, unpublished).

Jalil *et al.* (1997) determined the effect of temperature on mycelial growth of *T. harzianum* strain 39 (Trichodex 25% WP) and *B. cinerea* from tomato, in a thermogradient chamber from 0.36 to 39.30°C. *Trichoderma harzianum* showed initial mycelial growth at 7.7°C with an optimum at 26.8°C and a maximum of 35°C. In comparison, *B. cinerea* showed initial growth at 3.9°C, an optimum at 23.8°C and a maximum at 35°C. When both fungi were grown together at 20°C for 14 days, *T. harzianum* covered 69% of the Petri dish and *B. cinerea* only covered 31%.

*Gliocladium roseum* was found to control *B. cinerea* on strawberries (Peng & Sutton, 1991; Peng *et al.*, 1992; Sutton, 1995; Sutton *et al.*, 1997), raspberry (Yu & Sutton, 1997; Yu & Sutton, 1998; Sutton, *et al*, 1997), black spruce seedlings (Zhang *et al.*, 1994; Zhang *et al.*, 1996a; Zhang *et al.*, 1996b; Sutton *et al.*, 1997), and greenhouse crops (Sutton *et al.*, 1997). Because *B. cinerea* can survive endophytically (latently) on the grapevine, it should be possible to control the pathogen endophytically by means of *G. roseum* during the latent phase.

The saprophyte *Ulocladium atrum* was found to compete with *B. cinerea* on dead onion leaves (Köhl *et al.*, 1995a; Köhl *et al.*, 1995b; Köhl *et al.*, 1997), dead lily leaves (Köhl *et al.*, 1995c), necrotic strawberry stamens and dead strawberry leaves (Boff *et al.*, 1998), senescent cyclamen leaves (Köhl *et al.*, 1998) and to reduce the sporulation potential of the pathogen. *Botrytis cinerea* can colonise the dead, necrotic and aborted floral debris present in grape berry clusters early in the season that sporulate under conducive conditions to become a source of secondary inoculum in the vineyard to subsequently infect maturing grape berries later in the season. A reduction in the sporulation potential will lead to a reduction in the secondary inoculum and therefore to a reduction in *B. cinerea* decay of mature berries.

The optimum temperature for germination and mycelial growth for both *U. atrum* and *G. roseum* was found to be between 27 and 30°C on agar medium (Köhl *et al.*, 1999). *Ulocladium atrum* is less affected by lower temperature than *G. roseum*. At optimum temperature, 50% of conidia of *U. atrum* and *G. roseum* germinated within 2.6 and 10 hours, respectively. When the temperature was reduced to 6°C, the germination time increased to 18 and 96 hours, respectively. In bioassays conducted on dead onion leaves, *U. atrum* suppressed sporulation of *B. cinerea* and *B. aclada* by more than 85% from 6 to 24 °C. On dead cyclamen leaves, *G. roseum* was more efficient than *U. atrum* at 21°C and 24°C, but in contrast to *U. atrum*, showed no antagonistic activity below 21°C. On dead hydrangea leaves, *U. atrum* significantly reduced sporulation of *B. cinerea* at 3°C and 1°C.

Under Dutch growing conditions, the mean air temperature during leaf wetness periods in onion and lily fields is below 15°C and rarely above 20°C. In greenhouse crops the temperature is 17°C during high humidity periods. Köhl *et al.* (1999) concluded that *U. atrum* was better adapted to temperatures which occur in the field, the greenhouse crops or during cold storage than *G. roseum*.

The yeast *Trichosporon pullulans* was found to be effective in controlling *B. cinerea* on the grape berry surface late in the season and the potential of utilising the yeast as a post-harvest control measure is a possibility (Williamson, 1997).

## CONCLUSION

To control *B. cinerea,* growers rely heavily on fungicides. However, the development of *B. cinerea* strains resistant to fungicides has greatly reduced their effectiveness in disease control worldwide. This phenomenon, and the increasing public awareness concerning the negative effects of agrochemicals on the environment, emphasise the need for alternative products for disease control. Several attempts have been made to reduce *B. cinerea* in vineyards and in storage by means of biological control. However, the so called "silver bullet" approach in utilising a single antagonist, has its limitations when compared with synthetic fungicides (Spurr & Knudsen, 1985). Often the antagonist has a limited spectrum of activity and the duration of its effectiveness is less than that provided by synthetic fungicides. Furthermore, antagonists are more likely to be effective in preventing initial infection rather than resumption of a quiescent infection (Janisiewicz, 1988; Cook, 1993).

Therefore, due to the various points of infection utilised by *B. cinerea* and the fact that the pathogen can remain latent in the grapevine tissue, it may be possible to obtain effective control of the pathogen by using a number of different biological control agents each aimed at a different niche on the grapevine, protecting the plant at the various phenological stages of growth and under different microclimatic conditions as the season progresses.

In this study the ability of *T. harzianum*, *G. roseum* and *U. atrum* to colonise various niches on grapevine, and the possibility of controlling *B. cinerea* will be investigated. In addition the effect of combining these filamentous fungal antagonsits with the antagonistic yeast *Trichosporon pullulans* and fungicides will also be investigated.

## LITERATURE CITED

Aggenbach, W.A. & Marais, P.G. 1987. RP26019, a recently developed fungicide against *Botrytis cinerea* on wine grapes: disease control, residues, and effect on fermentation. *Phytophylactica* 10: 85-88.

Beever, R.E., Laracy, E.P. & Pak, H.A. 1989. Strains of *Botrytis cinerea* resistant to dicarboximide and benzimidazole fungicides in New Zealand vineyards. *Plant Pathology* 38: 427-437.

Beever, R.E., Pak, H.A. & Laracy, E.P. 1991. An hypothesis to account for the behaviour of dicarboximide-resistant strains of *Botrytis cinerea* in vineyards. *Plant Pathology* 40: 342-346.

Bessis, R. 1972. Étude de l'évolution des stomates et des tissus péeristomatiques du fruit de la vinge. *Comptes Rendus Academie Sciences (Paris)* 274: 2158-2161.

Boff, P., Geriaf, M. , Horsten, P., Lombaers-van der Plas, C.H. & Köhl, J. 1998. Sporulation suppression of *Botrytis cinerea* by *Ulocladium atrum* on strawberries. Abstract. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 2: 2.10.9.

Coertze, S. 1999. The behaviour of *Botrytis cinerea* on grape berries under different epidemiological conditions. MSc.Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Coertze, S. & Holz, G. 1999. Surface colonization, penetration, and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea*. *Plant Disease* 83: 917-924.

Coertze, S., Holz, G. & Sadie, A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*. *Plant Disease*. 85 : 668-667.

Cook, J. R. 1993. Making greater use of introduced micro-organisms for biological control of plant pathogens. *Annual Review of Phytopathology* 31: 53-80.

Creasy , L.L. & Coffee, M. 1988. Phytoalexin production potential of grape berries. *Journal of the Amercian Society for Horticultural Science* 113: 230-234.

De Kock, P.J. 1989. *Botrytis cinerea* bunch rot of table grapes: colonization and timing of fungicide applications. M.Sc.Agric. thesis, University of Stellenbosch, Stellenbosch South Africa.

De Kock, P. J. & Holz, G. 1991. Colonization of table grapes by *Botrytis cinerea* in the Western Cape province. *Phytophylactica* 23: 73-80.

De Kock, P.J. & Holz, G. 1994. Application of fungicides against postharvest *Botrytis cinerea* bunch rot of table grapes in the Western Cape. *South African Journal of Enology and Viticulture* 15: 33-40.

Delp, C.J. 1987. Benzimidazole and related fungicides. Pages 223-244 in: Modern Selective Fungicides – Properties, Applications, Mechanisms of Action. H. Lyr, ed., Longman, New York.

Doneche, B. 1986. La nature des exudates de raisins et leur role dans la germination des conidies de *Botrytis cinerea*. *Agronomie* 6: 67-73.

Edlich, W., Lorenz, G., Lyr, H., Nega, E. & Pommer, E.H. 1989. New aspects on the infection mechanism of *Botrytis cinerea* Pers. *Netherlands Journal of Plant Pathology* 95: (Supplement) 1:53-62.

English, J.T., Thomas, C.S., Marois, J.J. & Gubler, W.D. 1989. Microclimates of grapevine canopies associated with leaf removal and control of botrytis bunch rot. *Phytopathology* 79: 395-401.

Faretra, F. & Pollastro, S. 1993. Isolation, characterisation and genetic analysis of laboratory mutants of *Botryotinia fuckeliana* resistant to the phenylpyrrole fungicide CGA 173506. *Mycological Research* 97:620-624.

Fermaud, M. & Le Menn, R. 1992. Transmission of *Botrytis cinerea* to grapes by grape berry moth larvae. *Phytopathology* 82: 1393-1398.

Fourie, P.H. & Holz, G. 1998. Frequency of dicarboximide resistant strains of *Botrytis cinerea* in South African table grape vineyards and influence of spray schedules on resistant sub-populations. *South African Journal of Enology and Viticulture* 1: 3-9.

Fraile, A., Alonso, A. & Sagasta, E.M. 1986. Some characteristics of *Botrytis cinerea* isolates tolerant to procymidone. *Plant Pathology* 35: 82-85.

Gessler, C. & Jermini, M. 1985. Role of flower infections of grape by *Botrytis cinerea* and consequences for the spraying schedule. *Quaderni Della Scuola Di Specializzazione in Viticultura Ed Enologia, Università di Torino* 9: 245-250.

Gubler, W.D., Bettiga, L.J. & Heil, D. 1991. Comparisons of hand and machine leaf removal for the control of botrytis bunch rot. *American Journal of Enology and Viticulture* 42: 233-236.

Gubler, W.D., Marois, J.J., Bledsoe, A.M. & Bettiga, L.J. 1987. Control of botrytis rot of grape with canopy management. *Plant Disease* 71: 599-601.

Gullino, M.L. & Garibaldi, A. 1982. Use of mixture or alteration of fungicides with the aim of reducing the appearance of strains of *Botrytis cinerea*. *EPPO Bullitin* 12: 151.

Harman, G.E., Latorre, B., Agosin, E., San Martin, R., Riegel, D.G., Nielsen, P.A., Tronsmo, A. & Pearson, R.C. 1996. Biological and integrated control of Botrytis bunch rot of grape using *Trichoderma* spp. *Biological Control* 7: 259-266.

Harper, A.M., Strange, R.N., & Langcake, P. 1981. Characterization of the nutrients required by *Botrytis cinerea* to infect broad bean leaves. *Physiological Plant Pathology* 19: 153-167.

Harvey, J.M. 1955. A method of forecasting decay in California storage grapes. *Phytopathology* 45: 229-232.

Hill, G. 1985. Suberisation of cell walls: a defence reaction of grape stem tissue against invading mycelium of *Botrytis cinerea*. *Quaderni Della Scuola Di Specializzazione In Viticoltura Ed Enologia, Università di Torino* 9: 229-230.

Hill, G.K., Stellwaag-Kittler, F., Huth, G. & Schässer, E. 1981. Resistance of grapes in different development stages to *Botrytis cinerea*. *Phytopatologische Zeitschrift* 102: 329-338.

Holz, G. 1999. Behaviour and infection pathways of diverse fungal pathogens on fruit. Conference Handbook, 12th Biannual Australasian Plant Pathology Society Conference, Canberra, Australia, p 257 (Abstract).

Holz, G., Coertze, S. & Basson, E.J. 1997. Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. Annual Meeting of the American Phytopathological Society, Rochester, USA. *Phytopathology* 87: S88.

Holz, G., Coertze, S. & Basson, E.J. 1998. Infection pathways and latency of *Botrytis cinerea* in grape bunches. Abstract. 7th International Congress of Plant Patholology, Edinburgh, Scotland, Volume 3:6.22.

Hoos, G. & Blaich, R. 1988. Metabolism of stilbene phytoalexins in grapevines: oxidation of resveratrol in single-cell cultures. *Vitis* 21: 1-12.

Jalil, R.C., Norero, S.A. & Apablaza, H.G. 1997. Effects of temperature on mycelial growth of *Botrytis cinerea* and its antagonist *Trichoderma harzianum*. *Ciencia-e-Investigacion-Agraria* 24: 125-132.

Janisiewicz, W. 1988. Biological Control of Diseases of Fruits. Pages 153-165 in: Biocontrol of Plant Diseases Volume II. Muckerji, K. G. and Garg, K. L., eds. CRC Press, Inc. Boca Raton, Florida.

Jarvis, W. R. 1980. Epidemiology. Pages 219-250 in: The Biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic press.

Katan, T. 1982. Resistance to 3,5-dichlorophenyl-N-cyclic imide ('dicarboximide') fungicides in the grey mould pathogen *Botrytis cinerea* on protected crops. *Plant Pathology* 31: 133-141.

Köhl, J., Bélanger, R.R. & Fokkema, N.J. 1997. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: a comparative microscopic and ultrastructural study. *Phytopathology* 87: 634-642.

Köhl, J., Gerlagh, M., De Haas, B.H. & Krijger, M.C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. *Phytopathology* 88: 568-575.

Köhl, J., Molhoek, W.M.L., van der Plas, C.H. & Fokkema, N.J. 1995a. Suppression of sporulation of *Botrytis* spp. as valid biocontrol strategy. *European Journal of Plant Pathology* 101: 251-259.

Köhl, J., van der Plas, C.H., Molhoek, W.M.L. & Fokkema, N.J. 1995b. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. *European Journal of Plant Pathology* 101: 627-637.

Köhl, J., Molhoek, W.M.L., van der Plas, C.H. & Fokkema, N.J. 1995c. Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed o field conditions. *Phytopathology* 85: 393-401.

Köhl, J., van der Plas, C.H.L., Molhoek, W.M.L., Kessel, G.J.T. & van der Geijn, H.M.G. 1999. Competitive ability of the antagonists *Ulocladium atrum* and *Gliocladium roseum* at temperature favourable for *Botrytis* spp. development. *BioControl* 44: 329-346.

Kosuge, T. & Hewitt, W.B. 1963. Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea*. *Phytopathology* 54: 167-172.

Langcake, P. 1981. Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, ;-viniferin and pterostilbene. *Physiological Plant Pathology* 18: 213-226.

László, J.C., Combrink, J.C., Eksteen, G.J. & Truter, A.B. 1981. Effect of temperature on the emission of sulphur dioxide gas generators for grapes. *Deciduous Fruit Grower* 31: 112-119.

Latorre, B.A., Flores, V., Sara, M. & Roco, A. 1994. Dicarboximide-resistant isolates of *Botrytis cinerea* from table grape in Chile: survey and characterization. *Phytopathology* 78: 990-994.

Leroux, P. & Clerjeau, M. 1985. Resistance of *Botrytis cinerea* Pers. and *Plasmopara viticola* (Berk. & Curt.) Berl. and de Toni to fungicides in French vineyards. *Crop Protection* 4: 137-160.

Löcher, F.J., Lorenz, G. & Beetz, K.J. 1987. Resistance management strategies for dicarboximide fungicides in grapes: results of six years' trial work. *Crop Protection* 6: 139-147.

McClellan, W.D. & Hewitt, B. 1973. Early Botrytis rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. In *Vitis vinifera* L. *Phytopathology* 63: 1151-1157.

Nair, N.G. 1990. Strategies for fungicidal control of bunch rot of grapes caused by *Botrytis cinerea* in the Hunter valley. *Australian and New Zealand Wine Industry Journal* 5: 218-220.

Nair, N.G. & Hill, G.K. 1992. Bunch rot of grapes caused by *Botrytis cinerea*. Pages 147-169 in: Plant Diseases of International Importance. Vol III: Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

Nair, N.G. & Nadtotchei, A. 1987. Sclerotia of *Botrytis* as a source of primary inoculum for bunch rot of grapes in New South Wales, Australia. *Phytopathology* 119:42-51.

Nair, N.G. & Parker, F.E. 1985. Midseason bunch rot of grapes: an unusual disease phenomenon in the Hunter Valley, Australia. *Plant Pathology* 34: 302-305.

Nair, N.G., Guilbaud-Oulton, S., Barchia, I. & Emmett, R. 1995. Significance of carry over inoculum, flower infection and latency on the incidence of *Botrytis cinerea* in berries of grapevines at harvest in New South Wales. *Australian Journal of Experimental Agriculture* 35: 1177-1180.

Nelson, K.E. 1951. Effect of humidity on infection of table grapes by *Botrytis cinerea*. *Phytopathology* 41: 859-864.

Nelson, K.E. 1956. Effect of Botrytis infection on the tissue of Tokay grapes. *Phytopathology* 46: 223-229.

Northover, J. 1983. Segregation and parasitic fitness of isolates of *Botrytis cinerea* resistant to iprodione. *Canadian Journal of Plant Pathology* 5: 215-221.

Northover, J. 1987. Infection sites and fungicidal prevention of *Botrytis cinerea* bunch rot of grapes in Ontario. *Canadian Journal of Plant Pathology* 9: 129-136.

Northover, J. 1988. Persistance of dicarboximide-resistant *Botrytis cinerea* in Ontario vineyards. *Canadian Journal of Plant Pathology* 10: 123-132.

O'Neill, T.M., Elad, Y., Shtienberg, D. & Cohen, A. 1996. Control of grapevine grey mould with *Trichoderma harzianum* T39. *Biocontrol Science and Technology* 6: 139-146.

Padgett, M. & Morrison, J.C. 1990. Changes in the grape berry exudates during fruit development and their effect on the mycelial growth of *Botrytis cinerea. Journal of the American Society of Horticultural Sciences* 115: 269-273.

Peng, G. & Sutton, J.C. 1991. Evaluation of micro-organisms for biocontrol of *Botrytis cinerea* in strawberry. C*anadian Journal of Plant Pathology* 13: 247-257.

Peng, G., Sutton, J.C. & Kevan, P.G. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 14: 117-129.

Pezet, R. & Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans les grappes de *Vitis vinifera* (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18: 317-322.

Percival, D.C., Fisher, K.H. & Sullivan, J.A. 1994. Use of fruit zone leaf removal with *Vitis vinifera* L.cv. Riesling Grapevines. II. Effect on fruit composition, yield, and occurrence of bunch rot (*Botrytis cinerea* Pers.:Fr.). *American Journal of Enology and Viticulture* 45: 133-140.

Pommer, E.-H. & Lorenz, G. 1982. Resistance of *Botrytis cinerea* Pers. to dicarboximide fungicides - a literature review. *Crop Protection* 1: 221-230.

Pommer, E.-H. & Lorenz, G. 1995. Dicarboximide fungicides. Pages 99-111 in: Modern Selective Fungicides – Properties, Applications, Mechanisms of Action. H. Lyr, ed., Gustav Verlag, Jena.

Pucheu-Planté, B. & Mercier, M. 1983. Étude ultrastructurale de l'interrelation hôte-parasite entre le raisin et le champignon *Botrytis cinerea:* exemple de la pourriture noble en Sauternais. *Canadian Journal of Botany* 61: 1785-1797.

Savage, S.D. & Sall, M.A. 1982. The use of a radio-immunosorbent assay for *Botrytis cinerea. European Plant Protection Bulletin* 12: 49-53.

Smith, C.M. 1988. History of benzimidazole use and resistance. Pages 23-24 in: Fungicide resistance in North America. C.J. Delp, ed. APS Press, St. Paul, Minnesota.

Smith, V.L., Wilcox, W.F. & Harman, G.E. 1990. Potential for biological control of Phytopthora root and crown rots of apple by *Trichoderma* and *Gliocladium* spp. *Phytopathology* 80: 880-885.

Spotts, R.A. & Holz, G. 1996. Adhesion and removal of conidia of *Botrytis cinerea* and *Penicillium expansum* from grape and plum fruit surfaces. *Plant Disease* 80: 688-691.

Spurr, H.W. & Knudsen, G.R. 1985. Biological control of leaf diseases with bacteria. Pages 45-62 in: Biological Control Strategies on the Phylloplane. C.E. Windels and S. Lindow, eds. APS book, St. Paul, MN.

Stalder, I. 1953. Untersuchungen über die Graufäule. *Phytopathologische Zeitschrift* 22: 315-344, 345-380.

Stasz, T.E., Harman, G.E. & Weeden, N.F. 1988. Protoplast preparation and fusion in two biocontrol strains of *Trichoderma harzianum. Mycologia* 80: 141-150.

Sutton, J.C. 1995. Evaluation of micro-organisms for biocontrol: *Botrytis cinerea* and strawberry, a case study. Pages 173-190 in: Advances in Plant Pathology. Vol. II, Sustainability and Plant Pathology. J.H. Andrews and I.C. Tommerup, eds. Academic Press, London.

Sutton, J.C., Li, D.-W., Peng, G., Yu, H., Zhang, P. & Valdebenito-Sanhueza, R.M. 1997. *Gliocladium roseum,* a versatile adversary of *Botrytis cinerea* in crops. *Plant Disease* 81: 316-328.

Taylor, M.A., Chambers, K.R. & Watts, J.E. 1990. Sulphur dioxide damage as a predisposing factor to Botrytis rot of table grape berries. *Deciduous Fruit Grower* 40: 168-171.

Thomas, C.S., Marois, J.J. & English, J.T. 1988. The effects of wind speed, temperature, and relative humidity on development of aerial mycelium and conidia of *Botrytis cinerea* on grape. *Phytopathology* 78: 260-265.

Topolovec-Pintaric, S., Cvjetkovic, B. and Jurjevic, Z. 1999. Experience in integrated chemical-biological control of grey mould (*Botrytis cinerea*) on grapevines in Croatia. *Journal of Wine Research* 10: 33-41.

Vercesi, A., Locci, R. & Prosser, J.I. 1997. Growth kinetics of *Botrytis cinerea* on organic acids and sugars in relation to colonisation of grape berries. *Mycological Research* 101: 139-142.

Verhoeff, K. 1980. The infection process and host-pathogen interactions. Pages 153-180 in: The biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Williamson, L. 1997. Biocontrol of *Botrytis cinerea* on table grapes. MSc.Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Yu, H. & Sutton, J.C. 1997. Morphological development and interactions of *Gliocladium roseum* and *Botrytis cinerea* in raspberry. *Canadian Journal of Plant Pathology* 19: 237-246.

Yu, H. & Sutton, J.C. 1998. Effects of inoculum density, wetness duration, and temperature on control of *Botrytis cinerea* by *Gliocladium roseum* in raspberry. *Canadian Journal of Plant Pathology* 20: 243-252.

Zhang, P.G., Sutton, J.C. & Hopkin, A.A. 1994. Evaluation of micro-organisms for biocontrol of *Botrytis cinerea* in container-grown black spruce seedlings. *Canadian Journal of Forest Research* 24: 1312-1316.

Zhang, P.G., Sutton, J.C. & Hopkin, A.A. 1996a. Inoculum concentration and time of application of *Gliocladium roseum* in relation to biocontrol of *Botrytis cinerea* in black spruce seedlings. *Canadian Journal of Forest Research* 26: 360-367.

Zhang, P.G., Sutton, J.C., Tan, W. & Hopkin, A.A. 1996b. *Gliocladium roseum* reduces physiological changes associated with infection of black spruce seedlings by *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 18: 7-13.

Zoecklein, B.W., Wolf, T.K., Duncan, N.W., Judge, J.M. & Cook, M.K. 1992. Effects of fruit zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and White Riesling (*Vitis vinifera* L.) grapes. *American Journal of Enology and Viticulture* 43: 139-148.

# 2. FUNGICIDE COMPATIBILITY OF *GLIOCLADIUM ROSEUM*, *ULOCLADIUM ATRUM* AND *TRICHODERMA HARZIANUM* WITH FUNGICIDES COMMONLY APPLIED TO VINEYARDS

## ABSTRACT

Mycelial growth and germination tests were performed on agar in Petri dishes to determine the effect of fungicides, most commonly used in vineyards of the Western Cape province, on the growth of biocontrol agents *Trichoderma harzianum*, *Gliocladium roseum* and *Ulocladium atrum*. This was done to determine the use of these two control measures (chemical and biological) an integrated system. It was assumed that if the fungicide effectively inhibited the antagonist at 2.5 µg/ml active ingredient, the fungicide and antagonist could not be used in an integrated programme. Based on this criterium, *T. harzianum* can not be applied to vineyards with penconazole, mancozeb/metalaxyl, pyrifenox or mancozeb. In addition *T. harzianum* can not be applied as tank mixtures with iprodione. However, *T. harzianum* can be used in conjunction with pyrimethanil, folpet, iprodione, fosetyl-Al and copperhydroxide, provided the chemicals and the antagonist are applied alternately. *Gliocladium roseum* can not be applied in a tank mixture with pyrimethanil and penconazole, but can be used on grapevine in conjunction with penconazole, pyrifenox, pyrimethanil, iprodione and fosetyl-Al. *Ulocladium atrum* can not be applied with pyrimethanil and iprodione. *Ulocladium atrum* can be applied in conjunction with penconazole, pyrifenox, pyrimethanil, iprodione, fosetyl-Al and mancozeb. The fungus can be applied in a tank mixture with penconazole and pyrifenox.

## INTRODUCTION

Botrytis bunch rot, caused by *Botrytis cinerea* Pers.:Fr., is the major decay disease of stored grapes in the Western Cape province of South Africa (De Kock & Holz, 1991; 1994). To control this disease, growers rely heavily on fungicides, mainly dicarboximides (De Kock & Holz, 1994). However, the development of *B. cinerea* strains resistant to dicarboximides has greatly reduced the fungicide's effectiveness in disease control in South African table

grape vineyards (Fourie & Holz, 1998), as in many places around the world (Nair & Hill, 1992; Faretra & Pollastro, 1993; Smilanick, 1994). Resistance to fungicides, and the increasing public awareness concerning the negative effects of agrochemicals on the environment, emphasise the need for alternative products for disease control.

Several attempts have been made to reduce *B. cinerea* in vineyards and in storage by means of biological control (Ferreira, 1990; Sutton & Peng, 1993; McLaughlin *et al.*, 1990; Dubos, 1992; O'Neill *et al.*, 1996). However, the so called "silver bullet" approach in utilising a single antagonist, has its limitations when compared with synthetic fungicides (Spurr & Knudsen, 1985). Often the antagonist has a limited spectrum of activity and the duration of its effectiveness is less than that provided by synthetic fungicides. Furthermore, antagonists are more likely to be effective in preventing initial infection rather than resumption of a latent infection (Janisiewicz, 1988; Cook, 1993). Recent experiments have therefore concentrated on developing biological control which is integrated with cultural control practises (Gullino *et al.*, 1993), alternating biological and chemical control measures (Elad *et al.*, 1994; Elad *et al.*, 1995), and integrating different antagonistic micro-organisms exhibiting different modes of action (Falconi & Mendgen, 1994).

The potential of a biological approach to the reduction of *B. cinerea* on grapevine by non-antibiotic-producing antagonistic organisms, is currently being assessed in the Department of Plant Pathology, University of Stellenbosch, South Africa. Four organisms, known for their potential to control *B. cinerea,* are being investigated. They are *Trichoderma harzianum* (Elad, 1994; Harman *et al.*, 1996; O' Neill *et al.*, 1996; Latorre *et al.*, 1997), *Gliocladium roseum* (Peng *et al.*, 1992; Sutton *et al.*, 1997) and *Ulocladium atrum* (Köhl *et al.*, 1995; Köhl *et al.*, 1998). As the potential of the biological control agents will be investigated in an integrated system, the effect of various chemicals frequently applied to local vineyards on the organisms was investigated. Trials were conducted taking into account two possible scenarios. Firstly, the possible effect of fungicides applied to the vineyard after an application of the biological control agent or shortly before the application of the biocontrol agent. This entailed exposing the biocontrol agents to relatively low concentrations of the active ingredient of the fungicides, similar to the residue levels to which these organisms would be exposed under field conditions. Secondly, the possibility of applying the organisms and the fungicides at the same time, in other words by making use of spray mixtures in the

spray tank. This meant exposing the biocontrol agents to relatively high doses of the active ingredient of the various fungicides. All the trials investigating the effect of the fungicides on the biocontrol agents were done *in vitro* making use of Petri dish trials.

## MATERIALS AND METHODS

**Antagonists.** Unpatented isolates of the different biocontrol agents were used in the experiments. *Gliocladium roseum* was supplied by J.C. Sutton, University of Guelph, Ontario, Canada, and *Ulocladium atrum* by J. Köhl, DLO-Research Institute for Plant Protection, Wageningen, the Netherlands. *Trichoderma harzianum* and *T. pullulans* were obtained from J.H.S. Ferreira, ARC-Fruit, Vine and Wine Research Institute, Stellenbosch. Cultures of *G. roseum* and *T. harzianum* were maintained on potato dextrose agar (PDA) at 22°C. *Ulocladium atrum* was maintained on oatmeal agar (OMA, 20g oatmeal, 15g agar and 1L distilled water) (Köhl *et al.*, 1997) at 22°C. *Trichosporon pullulans* was cultivated on PDA amended with chloromycetin, incubated at 30°C in the dark.

**Fungicides.** A survey was conducted to determine which fungicides are most commonly used in vineyards of the Western Cape to control *Uncinula necator*, *Plasmopara viticola* and *Botrytis cinerea*. Based on the outcome of the survey (Table 1), eight fungicides were selected for use in the studies: penconazole (Topaz 20 EW, Novartis), pyrimethanil (Scala 40 SC, AgrEvo), folpet (Folpan 50 SC, Makhteshim-Agan), iprodione (Rovoral Flo 25 SC, Rhône-Poulenc), fosetyl-Al (Aliette 80 WG, Rhône-Poulenc), pyrifenox (Dorado 20 EC, Novartis), mancozeb (Sancozeb 80 WP, Sanachem) and mancozeb/metalaxyl (Ridomil MZ 60/10 WP, Novartis). The effect of copper hydroxide (Kocide 77 WP, Plaaskem) on *T. harzianum* was studied because copper hydroxide is one of the few chemicals allowed in organic viticulture (OASSA – Organic Viticulture Congress, South Africa, 1998). A stock solution of 1000 µg a.i./ml was prepared for each fungicide in a 100 ml glass container. A range of fungicide concentrations was then prepared from the stock solution. Molten potato dextrose agar (PDA, Biolab Co.) at approximately 50°C was amended with the various concentrations of the various fungicides and mixed with a vortex mixer. Media were poured into 90 mm sterile plastic Petri dishes.

**Mycelial growth test.** Petri dishes containing PDA amended with 0, 0.5, 2.5, 5.0 and 10.0 µg a.i./ml of the various fungicides, were inoculated with 5 mm agar plugs of the biocontrol agents. The agar plugs were taken from the active-growing margins of young pure cultures of *G. roseum* and *T. harzianum* cultivated on PDA and *U. atrum* cultivated on oatmeal agar (OMA) (20 g oatmeal, 15 g agar and 1 L distilled water) (Köhl *et al.,* 1997). Petri dishes were inoculated with three plugs each, arranged in a triangular pattern (three plates per concentration) and incubated at 22°C. *Trichoderma harzianum* colony size was measured after 2 days and *G. roseum* and *U. atrum* after 7 days. Each colony diameter was measured twice, at right angles. The diameter of the original mycelial plug was subtracted prior to analysis of the data.

**Germination test.** Spore suspensions were prepared by flooding active-growing young pure cultures in Petri dishes with sterile distilled water containing 0.01% Tween 80 and gently rubbing mycelial growth with a rubber spatula. The suspension was sonicated for 12 sec (three consecutive sonications of 4 s each) and filtered through two layers of sterile cheesecloth. The concentrations were determined with a heamocytometer and adjusted to 1 x $10^4$ conidia/ml with sterile distilled water containing 0.01% Tween 80 for *T. harzianum* and *G. roseum*. Spore suspensions for *U. atrum* were adjusted to 1 x $10^3$ conidia/ml. Petri dishes containing water agar (WA) amended with 0, 0.5, 2.5, 5.0 and 10.0 µg of the various fungicides (a.i.)/ml were inoculated with 1 ml of the conidial suspensions which was dispersed evenly over the plate with the aid of a sterile glass hockey stick. Dishes were allowed to dry in the laminar flow cabinet for 15 min, incubated at 22°C and germination was determined after 24 hours. Three microscopic fields were observed per plate under the 10 x magnification of the light microscope. Conidia were considered to have germinated if the germ tube was the at least the length of the conidium itself.

**Germination inhibition at spray tank concentrations.** The possibility of applying the organisms, and fungicides to which they reacted insensitive as spray tank mixtures, was tested in a seperate experiment. As the organisms would be used in tank mixtures primarily as conidia, conidia were exposed to a range of fungicide concentrations expected to occur in spray tanks (Table 2). A stock solution of 50 000 µg/ml was prepared for each fungicide in a 100 ml glass container, and a range of fungicide concentrations prepared as described previously. Petri dishes containing WA amended with 0, 25, 50, 5000 and 1000 µg a.i./ml of

the various fungicides were inoculated with 1 ml of the conidial suspensions which was dispersed evenly over the plate with the aid of a sterile glass hockey stick. Dishes were allowed to dry in the laminar flow cabinet for 15 min, incubated at 22°C and germination was determined after 24 hours.

**Statistical Analysis.** Percentage inhibition of mycelial growth and germination was determined relative to the control for each set of data.

## RESULTS

**Mycelial growth test.** Percentage mycelial inhibition for each organism exhibited by the different fungicides are given in Tables 3-5. Growth of the three organisms was reduced primarily by penconazole, iprodione and pyrifenox, although their sensitivities to the chemicals varied. *Trichoderma harzianum* (Table 3) was the most sensitive and the three fungicides at 2.5 µg/ml reduced mycelial growth by 92.9%, 82.8% and 95.0%, respectively. The organism was insensitive to the other chemicals, and a meaningful reduction in mycelial growth was not found at the highest concentration tested. *Gliocladium roseum* (Table 4) was less sensitive, and substantial reduction in growth was recorded at 2.5 µg/ml for iprodione, and at 5.0 µg/ml for penconazole and pyrifenox. *Ulocladium atrum* (Table 5) reacted similary to the three fungicides as *T. harzianum*: at 2.5 µg/ml mycelial growth was reduced by 68.5%, 77.9% and 77.1%, respectively. However, contrary to *T. harzianum,* the organism was marginally sensitive to pyrimethanil which reduced growth by 36.4% at 2.5 µg/ml.

**Germination test.** Percentage germination inhibition for each organism exhibited by the different fungicides are given in Tables 6-8. Mancozeb, macozeb/metalaxyl, penconazole and pyrifenox at 2.5 µg/ml completely inhibited spore germination of *T. harzianum* (Table 6). Mancozeb and macozeb/metalaxyl also effectively inhibited germination of *G. roseum* (Table 7) at 2.5 µg/ml. In the case of *U. atrum* (Table 8), mancozeb/metalaxyl was highly effective in inhibiting germination at 2.5 µg/ml, whereas mancozeb at 5.0 µg/ml caused only a 58.6% reduction in germination. Contrary to *T. harzianum* conidial germination of *G. roseum* (Table 7) and of *U. atrum* (Table 8) was not influenced by penconazole and pyrifenox. On the other hand, folpet inhibited germination of *G. roseum* by 98.4% at 2.5 µg/ml, where

folpet had little or no effect on *T. harzianum* (Tables 6 and 7). Folpet at 2.5 µg/ml also reduced germination of *U. atrum* by 42%.

**Germination inhibition at spray tank concentrations.** Iprodione reduced germination of *T. harzianum* by 95.4% at 25 µg/ml (Table 9), whereas inhibition was complete at spray tank concentration. The organism was unaffected by pyrimethanil at 50 µg/ml. No recordings could be done for this fungicide at spray tank concentration due to the high density of the medium. The organism was less sensitive to folpet, and at least 56% of the conidia germinated at 500 µg/ml which is higher than the tank concentration. Germination of *G. roseum* (Table 10) was completely inhibited by penconazole and pyrimethanil at 25 µg/ml, which is far below the tank concentration of the two fungicides. The organism was unaffected by iprodione at 50 µg/ml. No recordings could be done for this fungicide at spray tank concentration due to the high density of the medium. *Ulocladium atrum* (Table 11) was insensitive to penconazole and pyrifenox at tank concentration. Germination was however completely inhibited at tank concentration by pyrimethanil and iprodione.

## DISCUSSION

Residue levels on plant surfaces to which pathogens are exposed under field conditions normally range from approximately 1-10 µg/ml (Mitchell & Moore, 1962), whereas baseline values for fungicide sensitivity of most fungal poulations normally range from 0.03-3 µg/ml (Delp, 1988). According to these values, it can be assumed that if a fungicide inhibits the biological control agent at 2.5 µg/ml, the fungicide and the biological control agent can not be used in combination in an integrated disease control programme. Based on these assumptions, and the *in vitro* findings made in this study, *T. harzianum* can be used in conjunction with pyrimethanil, folpan, iprodione, fosetyl-Al and copperhydroxide, provided the chemicals and the antagonist are applied alternately. It is important to bear in mind that iprodione affects mycelial growth of *T. harzianum* severely, even at low concentrations. *Trichoderma harzianum* can not be applied to vineyards with penconazole, mancozeb/metalaxyl, pyrifenox or mancozeb. In addition *T. harzianum* can not be applied as tank mixtures with iprodione. *Gliocladium roseum* can be used in conjunction with penconazole, pyrifenox, pyrimethanil, iprodione and fosetyl-Al. Once again it is important to note that penconazole, iprodione and pyrifenox severly affect mycelial growth of this

antagonist at low concentrations. *Gliocladium roseum* can not be applied in a tank mixture with pyrimethanil and penconazole. *Ulocladium atrum* can be applied in conjunction with penconazole, pyrifenox, pyrimethanil, iprodione, fosetyl-Al and mancozeb (most likely). Once again it is important to note that penconazole, pyrimethanil, iprodione and pyrifenox severly affect mycelial growth of the antagonist. *Ulocladium atrum* can be applied in a tank mixture with penconazole and pyrifenox and can not be applied with pyrimethanil and iprodione.

Topolovec-Pintaric *et al.* (1999) found that Trichodex$^{T-39}$ can be mixed with fungicides containing the following active ingredients: cupric oxysulphate, cupric hydroxide, mancozeb, myclobutanil, dinocap, propineb, bifentine, phenualerate, alphametrine, sulphur, methoxydemeton, vamidotion, pyridaben and *Bacillus thuringiensis*. They also found that Trichodex$^{T-39}$ was inhibited by fenarimol, hexaconazole, metalaxyl/copper and propinconazole. Harman *et al.* (1996) found that conidial germination of *T. harzianum* strains P1 and 1295-22 was not inhibited by the fungicide iprodione, supporting the findings in my study. On the other hand, radial growth rate of both strains was severely affected by the presence of iprodione, which also supports my findings. Strain P1 was more inhibited than 1295-22, especially at low concentrations. They found that there was no increase in toxicity of the fungicide to either *T. harzianum* strain above 25 µg/ml. They theorised that this was most likely because the limit of solubility of the chemical was reached.

This study therefore showed that with careful management, the biocontrol agents can be applied in an integrated system with fungicides. The results reported here only reflect the effect of the fungicide on the specific isolate of the biocontrol agent used, and can not be applied to other isolates of the same species.

## REFERENCES

Cook, J. R. 1993. Making greater use of introduced micro-organisms for biological control of plant pathogens. *Annual Review of Phytopathology* 31: 53-80.

De Kock, P.J. & Holz, G. 1991. Colonization of table grapes by *Botrytis cinerea* in the Western Cape Province. *Phytophylactica* 23: 73-80.

De Kock, P.J. & Holz, G. 1994. Application of fungicides against postharvest *Botrytis cinerea* rot of table grapes in the Western Cape. *South African Journal of Enology and Viticulture* 15: 33-40.

Delp, C.J. 1988. Fungicide Resistance in North America. APS Press, St. Paul, Minnesota, USA.

Dubos, B. 1992. Biological control of *Botrytis*: state-of-the-art. Pages 169-178 in: Recent Advances in *Botrytis* Research. K. Verhoeff, N.E. Malathrakis and B. Williamson, eds. Pudoc, Wageningen.

Elad, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. *Crop Protection* 13: 35-38.

Elad, Y., Stienberg, D. & Niv, A. 1994. *Trichoderma harzianum* T39 integrated with fungicides: improved biocontrol of grey mould. *Proceedings of the Brighton Crop Protection Conference, Pests and Diseases* 3: 1109-1114.

Elad, Y., Gullino, M.L., Stienberg, D. & Aloi, C. 1995. Coping with tomato grey mould under Mediterranean conditions. *Crop Protection* 14: 105-109.

Falconi, C.J. & Mendgen, K. 1994. Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens *Botrytis cinerea*, *Monilinia fructigena* and *Penicillium expansum*. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 101: 38-47.

Faretra, F. & Pollastro, S. 1993. Isolation, characterisation and genetic analysis of laboratory mutants of *Botryotinia fuckeliana* resistant to the phenylpyrrole fungicide CGA 173506. *Mycological Research* 97: 620-624.

Ferreira, J.H.S. 1990. In vitro evaluation of epiphytic bacteria from table grapes for the suppression of *Botrytis cinerea*. *South African Journal of Enology and Viticulture* 11: 38-41.

Fourie, P.H. & Holz, G. 1998. Frequency of dicarboximide resistant strains of *Botrytis cinerea* in South African table grape vineyards and influence of spray schedules on resistant sub-populations. *South African Journal of Enology and Viticulture* 1: 3-9.

Gullino, M.L., Aloi, C., Mocioni, M. & Garibaldi, A. 1993. Biological and integrated control of grey mould in grapevine. Abstracts of the 6th International Congress of Plant Pathology, Montreal, Canada, p. 75.

Harman, G.E., Latorre, B., Agosin, E., San Martin, R., Riegel, D.G., Nielsen, P.A., Tronsmo, A. & Pearson, R.C. 1996. Biological and integrated control of *Botrytis* bunch rot of grape using *Trichoderma* spp. *Biological Control* 7: 259-266.

Janisiewicz, W. 1988. Biological control of diseases of fruits. Pages 153-165 in: Biocontrol of Plant Diseases, Volume II. Muckerji, K. G. and Garg, K. L., eds. CRC Press, Inc. Boca Raton, Florida

Köhl, J., van der Plas, C.H., Molhoek, W.M.L. & Fokkema, N.J. 1995. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. *European Journal of Plant Pathology* 101: 627-637.

Köhl, J., Bélanger, R.R. & Fokkema, N.J. 1997. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: a comparative microscopic and ultrastructural study. *Phytopathology* 87: 634-642.

Köhl, J., Gerlagh, M., De Haas, B.H. & Krijger, M.C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. *Phytopathology* 88: 568-575.

Latorre, B.A., Agosin, E., San Martin, R. and Vásquez, G.S. 1997. Effectiveness of conidia of *Trichoderma harzianum* produced by liquid fermentation against Botrytis bunch rot of table grape in Chile. *Crop Protection* 16: 209-214.

McLaughlin, R.J., Wisniewski, M.E., Wilson, C.L. & Chalutz, E. 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* spp. *Phytopathology* 80: 456-461.

Mitchell, J.E. & Moore, J.D. 1962. Relation of dodine residue levels and scab development on apple fruit and leaves. *Phytopathology* 52: 572-580.

Nair, N. G. & Hill, G. K. 1992. Bunch rot of grapes caused by *Botrytis cinerea*. Pages 147-169 in: Plant Diseases of International Importance. Vol III: Diseases of Fruit Crops. Kumar, J., Chaube, H. S., Singh, U. S. and Mukhopadhyay, A. N., eds. Prentice-Hall Inc., New Jersey.

Nel, A., Krause, M., Ramautar, N. & Van Zyl, K. 1999. A Guide for the Control of Plant Diseases. First edition. National Department of Agriculture, Pretoria.

O'Neill, T.M., Elad, Y., Shtienberg, D. & Cohen, A. 1996. Control of grapevine grey mould with *Trichoderma harzianum* T39. *Biocontrol Science and Technology* 6: 139-146.

Peng, G., Sutton, J.C. & Kevan, P.G. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 14:117-129.

Smilanick, J.L. 1994. Strategies for the isolation and testing of biocontrol agents. Pages 25-41 in: Biological Control of Postharvest Diseases, Theory and Practice. C. L. Wilson and M. E. Wisniewski, eds. CRC Press, Inc.

Spurr, H.W. & Knudsen, G.R. 1985. Biological control of leaf diseases with bacteria. Pages 45-62 in: Biological Control Strategies on the Phylloplane. C.E. Windels and S. Lindow, eds. APS book, St. Paul, MN.

Sutton, J.C. & Peng, G. 1993. Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Annual Review of Phytopathology* 31: 473-493

Sutton, J.C., Li, D.-W., Peng, G., Yu, H., Zhang, P. & Valdebenito-Sanhueza, R.M. 1997. *Gliocladium roseum,* a versatile adversary of *Botrytis cinerea* in crops. *Plant Disease* 81: 316-328.

Topolovec-Pintaric, S., Cvjetkovic, B. & Jurjevic, Z. 1999. Experience in integrated chemical-biological control of grey mould (*Botrytis cinerea*) on grapevines in Croatia. *Journal of Wine Research* 10: 33-41.

**Table 1.** Description of fungicides most commonly used on grapevine in the Western Cape province

| Trade name | Active ingredient | Chemical group | Target Pathogens |
|---|---|---|---|
| Aliette | Fosetyl-Al | Alkyl-phosphonate | Soil pathogens |
| Dorado | Pyrifenox | Pyridine | *Uncinula necator* |
| Folpan | Folpet | N-trihalomethylthio | *Botrytis cinerea, Plasmopara viticola, Phomopsis viticola* |
| Kocide | Copper hydroxide | Inorganic | *Botrytis cinerea* |
| Ridomil MZ | Mancozeb/metalaxyl | Dithiocarbamate/Phenylamide | *Plasmopara viticola* |
| Rovral | Iprodione | Dicarboximide | *Botrytis cinerea* |
| Sancozeb | Mancozeb | Dithiocarbamate | *Plasmopara viticola* |
| Scala | Pyrimethanil | Anilinopyrimidine | *Botrytis cinerea* |
| Topaz | Penconazole | Triazole | *Uncinula necator* |

**Table 2.** Active ingredients of fungicides and concentrations of active ingredients present in the spray tank

| Trade name | Active ingredient | Highest reommended dosage (/100L) [a] | Active ingredient in spray tank [b] |
|---|---|---|---|
| Dorado | Pyrifenox | 30.0 ml | 60 |
| Folpan | Folpet | 200.0 ml | 400 |
| Ridomil MZ | Mancozeb/Metalaxyl | 270.0 g | 600/100 |
| Rovral | Iprodione | 200.0 ml | 510 |
| Scala | Pyrimethanil | 120.0 ml | 480 |
| Topaz | Penconazole | 22.5 ml | 45 |

[a] Dosages recommended by Nel *et al.*, 1999.
[b] Expected concentration in spray tank.

**Table 3.** Growth[a] of *Trichoderma harzianum* on potato dextrose agar amended with fungicides at different concentrations

| | | Mycelial inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient** | **0.5** | **2.5** | **5.0** | **10.0** |
| Topaz | Penconazole | 75.0 | 92.9 | 93.9 | 96.2 |
| Scala | Pyrimethanil | 2.5 | 2.7 | 5.6 | 6.7 |
| Folpet | Folpan | 3.0 | 5.5 | 8.5 | 18.9 |
| Rovral | Iprodione | 24.0 | 82.8 | 85.2 | 76.4 |
| Aliette | Fosetyl-Al | -7.9 | -9.4 | -7.9 | -10.0 |
| Ridomil MZ | Mancozeb/Metalaxyl | 2.6 | 16.4 | 21.7 | 32.0 |
| Dorado | Pyrifenox | 73.2 | 95.0 | 95.1 | 93.5 |
| Sancozeb | Mancozeb | -4.6 | 0.0 | 7.9 | 15.8 |
| Kocide | Copperhydroxide | 9.1 | 6.4 | 0.7 | 6.2 |

[a] Colony diameters measured perpendicularly after 2 days growth at 22°C.

**Table 4.** Growth[a] of *Gliocladium roseum* on potato dextrose agar amended with fungicides at different concentrations

| | | Mycelial inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient** | **0.5** | **2.5** | **5.0** | **10.0** |
| Topaz | Penconazole | 17.5 | 53.5 | 66.2 | 74.8 |
| Scala | Pyrimethanil | -0.2 | 0.5 | 0.8 | -1.0 |
| Folpet | Folpan | -2.9 | 1.4 | 11.9 | 36.5 |
| Rovral | Iprodione | 24.0 | 82.8 | 85.2 | 76.4 |
| Aliette | Fosetyl-Al | -3.4 | -3.1 | -2.6 | -2.9 |
| Ridomil MZ | Mancozeb/Metalaxyl | 2.3 | 15.4 | 17.5 | 26.6 |
| Dorado | Pyrifenox | 13.8 | 44.3 | 56.3 | 72.6 |
| Sancozeb | Mancozeb | 0.3 | 7.6 | 13.3 | 18.5 |

[a] Colony diameters measured perpendicularly after 2 days growth at 22°C.

**Table 5.** Growth[a] of *Ulocladium atrum* on potato dextrose agar amended with fungicides at different concentrations

| | | Mycelial inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient** | **0.5** | **2.5** | **5.0** | **10.0** |
| Topaz | Penconazole | 12.4 | 68.5 | 85.3 | 95.5 |
| Scala | Pyrimethanil | 21.1 | 36.4 | 50.8 | 72.5 |
| Folpet | Folpan | -6.1 | 3.7 | 13.9 | 19.6 |
| Rovral | Iprodione | 19.9 | 77.9 | 76.9 | 80.4 |
| Aliette | Fosetyl-Al | -5.7 | -7.8 | -12.7 | -3.5 |
| Ridomil MZ | Mancozeb/Metalaxyl | 14.5 | 20.1 | 33.9 | 36.5 |
| Dorado | Pyrifenox | 36.2 | 77.1 | 91.7 | 100.0 |
| Sancozeb | Mancozeb | 8.5 | 17.4 | 16.2 | 30.6 |

[a] Colony diameters measured perpendicularly after 2 days growth at 22°C.

**Table 6.** Germination[a] of *Trichoderma harzianum* on water agar amended with fungicides at different concentrations

| | | Germination inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient** | **0.5** | **2.5** | **5.0** | **10.0** |
| Topaz | Penconazole | -1.5 | 100.0 | 100.0 | 100.0 |
| Scala | Pyrimethanil | -5.4 | -3.8 | -4.2 | -5.9 |
| Folpet | Folpan | -8.1 | 2.6 | 17.7 | 19.2 |
| Rovral | Iprodione | -7.3 | 0.6 | 0.4 | 25.3 |
| Aliette | Fosetyl-Al | -0.3 | -0.8 | -0.5 | -2.8 |
| Ridomil MZ | Mancozeb/Metalaxyl | 100.0 | 100.0 | 100.0 | 100.0 |
| Dorado | Pyrifenox | 5.0 | 100.0 | 100.0 | 100.0 |
| Sancozeb | Mancozeb | 38.5 | 100.0 | 100.0 | 100.0 |
| Kocide | Copperhydroxide | 1.2 | 1.7 | 2.3 | 2.2 |

[a] Germination determined after 24h incubation at 22°C.

**Table 7.** Germination[a] of *Gliocladium roseum* on water agar amended with fungicides at different concentrations

| | | Germination inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient** | **0.5** | **2.5** | **5.0** | **10.0** |
| Topaz | Penconazole | -2.7 | -2.4 | -1.9 | -1.3 |
| Scala | Pyrimethanil | -2.8 | -3.4 | -3.4 | 16.22 |
| Folpet | Folpan | -0.4 | 98.4 | 98.4 | 98.4 |
| Rovral | Iprodione | -1.4 | 11.7 | 6.6 | 98.4 |
| Aliette | Fosetyl-Al | 2.6 | 1.0 | 9.8 | 0.9 |
| Ridomil MZ | Mancozeb/Metalaxyl | 100.0 | 100.0 | 100.0 | 100.0 |
| Dorado | Pyrifenox | 2.7 | 7.0 | 2.8 | 58.0 |
| Sancozeb | Mancozeb | 69.5 | 100.0 | 100.0 | 100.0 |

[a] Germination determined after 24h incubation at 22°C.

**Table 8.** Germination[a] of *Ulocladium atrum* on water agar amended with fungicides at different concentrations

| | | Germination inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient** | **0.5** | **2.5** | **5.0** | **10.0** |
| Topaz | Penconazole | 0.4 | -0.4 | 1.0 | 0.6 |
| Scala | Pyrimethanil | 1.8 | -1.2 | 1.3 | 0.4 |
| Folpet | Folpan | -1.2 | 42.0 | 93.8 | 98.1 |
| Rovral | Iprodione | 0.3 | 3.7 | 8.5 | 8.0 |
| Aliette | Fosetyl-Al | -0.3 | 0.4 | -0.3 | 2.0 |
| Ridomil MZ | Mancozeb/Metalaxyl | -1.1 | 100.0 | 100.0 | 100.0 |
| Dorado | Pyrifenox | 0.9 | 0.9 | 2.0 | 1.9 |
| Sancozeb | Mancozeb | 0.0 | -1.7 | 58.6 | 100.0 |

[a] Germination determined after 24h incubation at 22°C.

**Table 9.** Germination[a] of *Trichoderma harzianum* on water agar amended with fungicides at different concentrations

| | | Germination inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient[b]** | **25** | **50** | **500** | **1000** |
| Scala | Pyrimethanil (480) | 1.5 | 8.9 | NR[c] | NR |
| Folpet | Folpan (400) | 31.3 | 32.6 | 44.5 | 75.7 |
| Rovral | Iprodione (510) | 95.4 | 95.7 | 100.0 | 100.0 |

[a] Germination determined after 24h incubation at 22°C.

[b] Expected concentration in spray tank.

[c] NR = No Reading due to opaque medium.

**Table 10.** Germination[a] of *Gliocladium roseum* on water agar amended with fungicides at different concentrations

| | | Germination inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient[b]** | **25** | **50** | **500** | **1000** |
| Topaz | Penconazole (45) | 100.0 | 100.0 | 100.0 | 100.0 |
| Scala | Pyrimethanil (480) | 100.0 | 100.0 | NR[c] | NR |
| Rovral | Iprodione (510) | 4.9 | 1.8 | NR | NR |

[a] Germination determined after 24h incubation at 22°C.
[b] Expected concentration in spray tank.
[c] NR = No Reading due to opaque medium.

**Table 11.** Germination[a] of *Ulocladium roseum* on water agar amended with fungicides at different concentrations

| | | Germination inhibition (%) relative to control | | | |
|---|---|---|---|---|---|
| | | Concentration (µg/ml) | | | |
| **Fungicide** | **Active ingredient[b]** | **25** | **50** | **500** | **1000** |
| Topaz | Penconazole (45) | -3.9 | -1.7 | 100.0 | 100.0 |
| Scala | Pyrimethanil (480) | 6.1 | 32.7 | 96.2 | 100.0 |
| Rovral | Iprodione (510) | 90.1 | 97.3 | 100.0 | 100.0 |
| Dorado | Pyrifenox (60) | -3.5 | 4.2 | 8.9 | 100.0 |

[a] Germination determined after 24h incubation at 22°C.
[b] Expected concentration in spray tank.

# 3. COLONISATION OF SITES IN CHARDONNAY GRAPE BUNCHES BY POTENTIAL BIOCONTROL ORGANISMS, AND THE OCCURRENCE OF *BOTRYTIS CINEREA*

## ABSTRACT

The potential of three fungal antagonists (*Gliocladium roseum, Ulocladium atrum* and *Trichoderma harzianum*) and one yeast (*Trichosporon pullulans*) to reduce *Botrytis cinerea* infection in grape bunches, was investigated in a commercial vineyard planted with the wine grape cultivar Chardonnay. Antagonists were applied as conidial suspensions to bunches at various phenological stages. Bunches were collected 2 wk after application, surface-sterilised and used for determining antagonist colonisation and *B. cinerea* infection at specific sites in the bunches. The antagonists colonised the different sites, but colonisation during the three seasons was inconsistent and sporadic. *Ulocladium atrum* and *G. roseum* colonised floral debris to a degree in the 1996 season. However, in the 1997 season these two antagonists did not develop from floral debris. *Trichoderma harzianum* colonised floral debris extensively in the 1996 season. In the 1997 season colonisation by *T. harzianum* dropped, but unlike *G. roseum* and *U. atrum*, *T. harzianum* occurred at a low level in flowers. *Ulocladium atrum* only colonised bunches during bloom, and was not found in bunches monitored from pea-size stage to véraison. This finding suggests that the saprophyte colonised moribund and dead flower parts occurring in bunches during full bloom to the pre-pea-size stage, and is not likely to be found in living tissue. *Gliocladium roseum* colonised grape berries and pedicels to some degree and *T. harzianum* colonised these grape parts extensively. *Botrytis cinerea* occurred inconsistently, and at low frequencies in the different sites in bunches. It was therefore not possible to comment on the effectivity of the various antagonists in the three seasons during which the trials were performed. The findings however indicated that climatic conditions occurring in vineyards in the Western Cape province are not well suited for the establishment of the isolates of the biocontrol agents tested in this study.

# INTRODUCTION

*Botrytis cinerea* Pers.:Fr., a pathogen of grapevine (*Vitis vinifera* L.), is associated with early-season infection (McClellan & Hewitt, 1973; Nair, 1985; Nair & Parker, 1985) and infection of mature grapes favoured by late-season rains or prolonged periods of high relative humidity (Harvey, 1955; Jarvis, 1980). Infection of immature berries is often followed by a latent period, defined as the interval from infection to the display of macroscopic symptoms (McClellan & Hewitt, 1973). Evidence for the importance of these latent infections in subsequent disease development is primarily circumstantial. In California and Australia, McClellan and Hewitt (1973) and Nair and Parker (1985) found that berry infection takes place during bloom. They showed that *B. cinerea* invades the stigma and style and then becomes latent in necrotic stigma and style tissue at the style end of the berry. Grape clusters remain symptomless between the flowering period and the beginning of ripening, and a pathogenic relationship is generally established once the fruit ripens. At véraison or later the fungus resumes growth and rots the berry (Mclellan & Hewitt, 1973; Nair & Parker, 1985; Pezet & Pont, 1986). In Switzerland, Pezet and Pont (1986) found no evidence for the style end infection pathway and showed that latent infection was predominantly pedicel-associated. Their histological studies of laboratory-inoculated bunches showed that *B. cinerea* colonises the stamens during bloom and invades their base situated on the receptacle. From there it spreads to the pedicel and via the vascular tissue into the berries. Savage and Sall (1982), however, were unable to detect the pathogen in immature berries. It was later shown (Holz *et al.*, 1997, 1998; Holz, 1999) that berry cheeks were virtually free from natural *B. cinerea* infection during all developmental stages, and confirmed that berry infection was predominantly pedicel-associated. These workers (Holz *et al.*, 1997, 1998; Holz, 1999; Gütschow, 2001) furthermore showed that natural latent *B. cinerea* infection generally occurs in the other morpohological parts of grape bunches, and is therefore not exclusive to the grape pistil. Disease management strategies should therefore concentrate on the protection of the internal bunch parts against infection by *B. cinerea*.

To control this disease in the Western Cape province of South Africa, growers rely heavily on fungicides, mainly dicarboximides (De Kock & Holz, 1991; 1994). However, the development of *B. cinerea* strains resistant to dicarboximides has greatly reduced the fungicide's effectiveness in disease control in South African table grape vineyards (Fourie & Holz, 1998), as in many places around the world (Nair & Hill, 1992; Faretra & Pollastro,

1993; Smilanick, 1994). Resistance to fungicides, and the increasing public awareness concerning the negative effects of agrochemicals on the environment, emphasise the need for alternative products for disease control. Several attempts have been made to reduce *B. cinerea* in vineyards and in storage by means of biological control (Ferreira, 1990; Sutton & Peng, 1993; McLaughlin *et al.*, 1990; Dubos, 1992; O'Neill et al., 1996). However, the so called "silver bullet" approach in utilising a single antagonist, has its limitations when compared with synthetic fungicides (Spurr & Knudsen, 1985). Often the antagonist has a limited spectrum of activity and the duration of its effectiveness is less than that provided by synthetic fungicides. Furthermore, antagonists are more likely to be effective in preventing initial infection rather than resumption of a latent infection (Janisiewicz, 1988; Cook, 1993). Therefore, due to the various infection sites in grape bunches utilised by *B. cinerea* and the fact that the pathogen can remain latent in the grapevine tissue, it may be possible to obtain effective control of the pathogen by using different biological control agents each aimed at a different site in the bunch, protecting the bunch at the various phenological stages of growth and under different microclimatic conditions.

The possibility of biological control of *B. cinerea* with *Trichoderma harzianum* has been investigated by a number of researchers (Elad, 1994; Harman *et al.*, 1996; O'Neill *et al.*, 1996; Latorre *et al.*, 1997). A biocontrol product, based on *T. harzianum* Rifai (T-39) and marketed in several countries as Trichodex 25 P (Makhteshim Chemical Works), is the first biocontrol agent for the control of *B. cinerea* on grapevine to be used commercially (Elad *et al.*, 1994). *Gliocladium roseum* was found to control *B. cinerea* on strawberries (Peng & Sutton, 1991; Peng *et al.*, 1992; Sutton, 1995; Sutton *et al.*, 1997), raspberry (Yu & Sutton, 1997; Yu & Sutton, 1998; Sutton, *et al*, 1997), black spruce seedlings (Zhang *et al.*, 1994; Zhang *et al.*, 1996a; Zhang *et al.*, 1996b; Sutton, *et al.*, 1997), and greenhouse crops (begonia, cyclamen, geranium, cucumber, pepper and tomato), (Sutton, *et al*, 1997). Because *B. cinerea* can survive endophytically (latently) on the grapevine, it should be possible to control the pathogen endophytically by means of *G. roseum* during the latent phase. The saprophyte *Ulocladium atrum* was found to compete with *B. cinerea* on dead onion leaves (Köhl *et al.*, 1995a; Köhl *et al.*, 1995b; Köhl *et al.*, 1997), dead lily leaves (Köhl *et al.*, 1995c), necrotic strawberry stamens and dead strawberry leaves (Boff *et al.*, 1998), senescent cyclamen leaves (Köhl *et al.*, 1998) and to reduce the sporulation potential of the pathogen. *Botrytis cinerea* can colonise the dead, necrotic and aborted floral debris present in grape berry clusters early in the season and sporulate under conducive conditions to become a

source of secondary inoculum in the vineyard to subsequently infect maturing grape berries later in the season. A reduction in the sporulation potential will lead to a reduction in the secondary inoculum and therefore to a reduction in decay of mature berries. The yeast *Trichosporon pullulans* was found to be effective in controlling *B. cinerea* on the grape berry surface late in the season (Williamson, 1997).

This study reports on an investigation into the possibility of controlling *B. cinerea* on the wine grape cultivar, Chardonnay, by investigating the colonisation under field conditions of different sites in bunches by three fungal antagonists (*Gliocladium roseum, Ulocladium atrum* and *Trichoderma harzianum*) and one yeast (*Trichosporon pullulans*).

## MATERIALS AND METHODS

**Vineyards.** Experiments were conducted during the 1996/97-1998/99 growing seasons in experimental plots in a vineyard of the wine grape cultivar Chardonnay at the ARC – Fruit, Vine and Wine Research Institute, Stellenbosch. This vineyard was chosen because of the high incidence of naturally occurring *B. cinerea* and due to the high susceptibility of this cultivar to *B. cinerea*. Vineyard blocks ranged from 1-5 ha and the vines were trained to a two wire trellis system. All vines were drip-irrigated. During the 1996/97 growing season the recommended program for the control of downy and powdery mildew was not followed. This was done because the effect of these fungicides on the antagonists had not yet been determined. In the 1997/98 and 1998/99 seasons the normal downy and powdery mildew programs were followed, taking into account the results reported in Part 2.

**Antagonists.** Unpatented isolates of the different biocontrol agents were used in the experiments. *Gliocladium roseum* was supplied by J.C. Sutton, University of Guelph, Ontario, Canada, and *Ulocladium atrum* by J. Köhl, DLO-Research Institute for Plant Protection, Wageningen, the Netherlands. *Trichoderma harzianum* and *T. pullulans* were obtained from J.H.S. Ferreira, ARC-Fruit, Vine and Wine Research Institute, Stellenbosch. Cultures of *G. roseum* and *T. harzianum* were maintained on potato dextrose agar (PDA) at 22°C. *Ulocladium atrum* was maintained on oatmeal agar (OMA, 20g oatmeal, 15g agar and 1L distilled water) (Köhl *et al.*, 1997) at 22°C. *Trichosporon pullulans* was cultivated on PDA amended with chloromycetin, incubated at 30°C in the dark.

Inocula of the antagonists were prepared on different grain media. *Gliocladium roseum* was propagated on wheat grains in 1 L consol glass jars (J.C. Sutton, personal communication). Each jar contained 250 g of wheat with an equal volume of distilled water. Jars were steamed for 3 h and allowed to stand overnight to allow contaminating bacteria and yeasts to germinate before the final sterilisation process. Jars containing steamed wheat were autoclaved for 20 min at 120°C. Water remaining in the jars was poured out in a laminar flow cabinet before inoculation of the wheat. Once the wheat had cooled down each jar was inoculated with 10 ml of a *G. roseum* spore suspension containing 1 x $10^7$ conidia/ml. The jars were kept at 22°C and the lids opened every 4-5 days to allow an exchange of air. Jars were shaken every 1-2 days and after 2 wk, the metal lids were replaced with sterilised filter paper discs to allow slow drying of the wheat, which facilitates high levels of spore production (Zhang *et al.*, 1996a). Once spore production began, the jars were exposed to black light to accelerate spore production. After 30 days the grains were covered in a mass of light pinkish, orange conidia.

*Ulocladium atrum* was propagated on oat grains in 250 ml Erlenmeyer flasks according to the method used by Köhl *et al.* (1995c). Each flask contained 30 g oats and an equal volume of distilled water. Flasks were sealed with cotton wool plugs covered in aluminum foil. The oat grains were steamed and sterilised in the same way as the wheat. Each flask was inoculated with 5 ml of a *U. atrum* spore suspension containing 1 x$10^7$ conidia/ml. Flasks were shaken every 2-4 days and incubated at 18°C in the dark for 28 days.

*Trichoderma harzianum* was propagated on wheat grains. One liter Erlenmeyer flasks were filled with 30 g of wheat and an equal volume of distilled water. Wheat grains were sterilised by autoclaving at 120°C for 20 min, twice, consecutively. Once the grains had cooled down they were inoculated with a *T. harzianum* spore suspension containg 1 x $10^7$ conidia/ml. Flasks were incubated for 2 wk at 22°C under normal light and shaken periodically.

Fungal spore suspensions were prepared by suspending the colonised grains in sterilised, distilled water containing 0.01% Tween 80, and placed on a rotary shaker for 30 min. The suspension was filtered through a double layer of sterile cheesecloth. Concentrations of spore suspensions were determined with a heamocytometer and adjusted to 1 x $10^6$ conidia/ml with sterile distilled water containing 0.01% Tween 80. Germination was estimated on PDA for

*G. roseum* and *T. harzianum* and on OMA for *U. atrum.* Germination consistently exceeded 95% for all three antagonists.

*Trichosporon pullulans* was propagated in 1000 ml Nutrient Yeast Dextrose Broth (NYDB, 8g nutrient broth, 5g yeast extract and 10g D-glucose, suspended in one liter distilled water) in 2 L Schott bottles. The medium was inoculated with yeast cells and placed in a rotary incubator at 180 rpm for 20 h at 30°C. A yeast cell suspension was prepared by centrifuging yeast medium at 8000 rpm for 5 min at 20°C. The supernatant was poured off and the yeast cells resuspended with a 0.7% salt solution. Concentrations were estimated from standard curves for cfu/ml suspension and absorbance at 630 nm on a spectrophotometer (Ultraspec 2000 [U/V Visible Spectrophotometer] Pharmacia Biotech). Concentrations were adjusted to 1 x $10^7$ cfu/ml with a 0.7% salt solution.

**Spray Programmes.** During the 1996/97 and 1997/98 seasons, the fungal antagonists were applied in combination with the yeast, and the fungicide standard iprodione (Rovral Flo 25 SC, Rhône Poulenc Agrichem) (Table 1). In the 1998/1999 season the fungal antagonist found most effective in the previous seasons was applied in combination with the yeast, and with two fungicide standards iprodione (Rovoral Flo 25 SC, Rhône Poulenc Agrichem) and pyrimethanil (Scala SC 40, AgrEvo) (Table 2). Control treatments consisted of water plus 0.01% Tween 80. Treatments were applied to single-row plots, each consisting of six mature vines. Rows used in the experiment were separated from the commercial vines by untreated buffer rows. Each treatment was conducted as a completely randomised design with four replicates in the first two seasons and eight replicates in the last season. Treatments were applied with hand held equipment until runoff.

**Colonisation of bunch parts by antagonists and *B. cinerea*.** Two methods were used to determine the ability of antagonists to colonise the different sites in grape bunches, and to suppress natural infection by the pathogen. In the first method, which was conducted only during bloom, bunches were collected at the pre-pea-size stage 14 days after the application at bloom. Moribund and dead flower parts were removed from the bunches, placed on Kerssies *B. cinerea* selective medium (Kerssies, 1990) in Petri dishes and incubated at 22°C under diurnal light. The bunches were divided in sections bearing a short section of the rachis, laterals and eight-nine berries. The sections were surface-sterilised (10 s 70% ethanol, 1 min 0.35% sodium hypochlorite, 1 s 70% ethanol) to kill the fungi on the surface, placed on

paraquat-chloramphenicol agar (PQCA, 1L distilled water containing 10g Agar was autoclaved and allowed to cool, and 20 mg a.i. paraquat and 200 mg chloramphenicol were added) (Peng & Sutton, 1991) in Petri dishes and incubated at 22°C under diurnal light. Preliminary studies showed that the selective media had no effect on the antagonists. The material was regularly examined for the development of the antagonists and *B. cinerea*. The percentage sites in a bunch section yielding an antagonist or the pathogen were recorded after 14 days.

In the second method, which was conducted from pea-size stage to véraison, bunches were collected 14 days after antagonist application. The bunches were divided in sections and surface-sterilised as described previously. The sections were immersed in a paraquat solution (30 ml paraquat in 1 L water for 30 sec) to terminate host resistance (Gindrat & Pezet, 1994). The sections were placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2cm) in ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). The chambers were kept at 22°C under a diurnal light regime (12 h photoperiod) and the sections were regularly examined for the development of the antagonists and *B. cinerea*. The percentage sites in a bunch section yielding an antagonist or the pathogen were recorded after 14 days.

**Climatic conditions.** Temperature and rainfall for the 1996-1998 growing seasons were recorded at weather stations at the ARC-Fruit, Vine and Wine Research Institute, Stellenbosch. Periods conducive to *U. atrum* and *G. roseum* were determined according to Köhl *et al.* (1999). For both organisms optimum growth occurs at 27 – 30°C and long wetness periods. Optimum conditions for colonisation by *T. harzianum* is at 26.8°C and long wetness periods (Jalil *et al.*, 1997). Periods conducive to *B. cinerea* infection during each growing season were determined on the basis of the infection criteria of Sall *et al.* (1981). A rainy period was considered conducive to the natural development of *B. cinerea* if more than 5 mm rain was recorded during 24 h (relative humidity ≥92%; average temperature 15-22°C), or if 1-5 mm rain fell on each of two consecutive days (relative humidity ≥92%; average temperature 15-22°C).

**Statistical Analysis.** The experiments were arranged in a completely randomised design. Statistical computations were performed using the SAS (SAS Institute Inc., Cary, NC). Data

was tested for normality and if necessary a suitable transformation was performed on the data. All the data was examined using analysis of variance (ANOVA) and treatment means were compared using the Student's *t*-test (Snedecor and Cochran, 1980).

## RESULTS

**Climatic conditions conducive to antagonist development.** Daily temperature and rainfall for the 1996-1999 growing seasons are shown in Fig. 1. Climatic conditions ranged from cool and wet during the first season to hot and dry for the last season.. Average temperatures for the three consecutive growing seasons were 18.38, 19.71 and 20.16°C, respectively, whereas total rainfall of 240.20, 177.40 and 126.20 mm rain was recorded. In 1996, rain events during the 3-day period prior to antagonist application which might lead to humid conditions in vineyards on the day of antagonist application, occurred during bloom. In 1997, a conducive period was recorded prior to the application at pea-size stage. In 1998, no rain was recorded during the 3-day period prior to any of the antagonist application dates. Mean temperatures and rainfall during the 24 h-period following the antagonist application, are given in Table 3-5. According to these data, it was generally dry and prevailing temperatures were much lower than the optimum temperatures needed for maximum development by the antagonsists.

**Colonisation by antagonists of moribund and dead flower parts.** *Trichoderma harzianum* displayed high colonisation of floral debris (Table 6). However, levels at which colonisation occurred fluctuated drastically between seasons. In the 1996 season, 100% of the stamens, calyptra and embryos obtained from bunches in the *T. harzianum* programmes yielded the organism (Table 6). In 1997, 10-18% of stamens, 27-30% of calyptra and 25-50% of embryos yielded the antagonist. However, in the 1998 season colonisation by *T. harzianum* dropped and the organism developed only from the embryos (Table 7). *Ulocladium atrum* (Table 6) and *G. roseum*, on the other hand, displayed poor colonisation and developed erratically and at low levels from the different tissues in 1996. None of the floral debris yielded the organisms in the 1997 season.

**Colonisation by antagonists of living bunch parts.** Percentages at which the different bunch tissues yielded the antagonists during the various stages of bunch growth are given in Tables 8-14. The colonisation of the antagonists are summarised below. *Trichoderma*

*harzianum* displayed, depending on the season, the highest colonisation of living tissue. Overall, the organism occurred at maximum levels from the different sites after the pea-size and bunch closure applications (Table 9-12). *Trichoderma harzianum* furthermore showed preference for colonising primarily the pedicels and berries. In the 1996/1997 growing season, 100% of the pedicels and berries yielded the organism after the pea size and bunch closure applications. Colonisation by *T. harzianum* dropped slightly on pedicels in the 1997/1998 season and drastically on berries. In the 1998/1999 season colonisation was poor and *T. harzianum* developed erratically from the different sites in the bunches. *Gliocladium roseum*, on the other hand, displayed poor colonisation and developed erratically and at low levels from the different tissues at each sampling. An exception was found during bloom, when the young tissues yielded *G. roseum* more consistently (Table 8). The saprophyte *U. atrum*, as expected, did not develop from bunch parts sampled after the pea size to vérasion stages. The organism was however found at high levels in bunches sampled after the full bloom treatment (Table 8).

**Climatic conditions conducive to *B. cinerea*.** The number of *B. cinerea* infection periods recorded before each sampling are given in Table 15. In the 1996/1997 season, climatic conditions favoured the natural development of *B. cinerea* from bloom to bunch closure. Thereafter, conditions were generally unfavourable for the development of the pathogen. In 1997/1998 season, conducive periods were recorded only during the early and late pea-size stage. In 1998/1999 season, climatic conditions favoured the natural development of *B. cinerea* only during bloom.

**Occurrence of *B. cinerea* in moribund and dead flower parts.** The pathogen occurred inconsistently, and in low frequencies in flower parts removed from bunches obtained from the various programmes. In both 1996 and 1997, none of the tissues from the control, and from programmes 2, 3, 4, 6, 7, 8 and 9 yielded the pathogen (Table 16). The pathogen developed only from bunches treated with *G. roseum* and *T. harzianum*, but occurred eratically in the tissues. In 1998, *B. cinerea* was regularly found in flowers from bunches of all treatments (Table 17). Levels were low in the stamens and calyptra, but ranged from approximately 6-25% in embryos. However, incidences in tissues from the different treatments did not differ significantly.

**Occurrence of *B. cinerea* in living bunch parts.** The occurrence of the pathogen in sites in bunches at various stages of bunch growth are given in Tables 18-24 and is summarised below. In both 1996 and 1997, *B. cinerea* incidences in pre-pea size bunches were low and levels in berries and rachises did not differ significantly between treatments. In each season in nearly all treatments, *B. cinerea* consistently developed from pedicels of bunches at pea size and bunch closure stages (Table 19-20). Incidences of pedicel infection at these growth stages were generally high in the 1996 season, and low in the following seasons. Levels of pedicel infection generally reached a maximum at pea-size and bunch closure stages, and then declined. Berries in bunches of the different treatments sporadically yielded *B. cinerea*, and incidences were generally lower than in pedicels. Furthermore, in nearly all the infected berries, the pathogen developed from the pedicel-end of the berry. The pathogen occasionally developed from rachises of bunches sampled from bloom to bunch closure, but was absent in rachises of bunches sampled during véraison.

**Suppression of *B. cinerea* colonisation of bunch parts.** At each phenological stage in each season, *B. cinerea* was less commonly associated with bunches from treatments that received *T. harzianum* than with bunches in the other treatments. This tendency was most evident in both the 1996/1997 and 1997/1998 seasons at pea size stage in pedicels (Tables 19-20), the preferential site for colonisation by *B. cinerea*.

## DISCUSSION

The study showed that *U. atrum*, *G. roseum*, *T. harzianum* and *T. pullulans* could each contribute to the reduction of *B. cinerea* in grape bunches by colonising the different sites available to the pathogen. However, the occurrence of the antagonists in the different bunch sites fluctuated drastically between seasons. *Ulocladium atrum* and *G. roseum* colonised floral debris to a degree in the 1996 season. However, in the 1997 season these two antagonists were not found in floral debris. *Trichoderma harzianum* colonised floral debris extensively in the 1996 season. In the 1997 season colonisation by *T. harzianum* dropped, but unlike *G. roseum* and *U. atrum*, *T. harzianum* occurred in flowers at low levels. *Ulocladium atrum* only colonised bunches during bloom, and was not found in bunches monitored from pea-size stage to véraison. This finding suggests that the saprophyte colonised moribund and dead flower parts occurring in bunches during full bloom to the pre-pea size stage, and is not likely to be found in living tissue. *Gliocladium roseum*, on the other

hand, displayed poor colonisation of the sites in developing bunches, and developed erratically and at low levels from the different tissues at each sampling. An exception was found during bloom, when the young tissues yielded *G. roseum* more consistently. *Trichoderma harzianum* displayed, depending on the season, the highest colonisation of the different sites in developing bunches.

The phenomenon that the antagonists were sporadically found in bunches, and inconsistently in the different sites, indicated that climatic conditions occurring in vineyards in the Western Cape province are not well suited for the establishment of the isolates of the biocontrol agents tested in this study. The optimum temperature for *U. atrum* and *G. roseum* is between 27 – 30°C (Köhl *et al.,* 1999) and for *T. harzianum* it is 26°C (Jalil *et al.,* 1997). Temperatures recorded in the vineyard on the day after antagonist application were much lower than these optimum temperatures. Furthermore, no rain fell on the day before or after antagonist application. In spite of this, *T. harzianum* colonised the grapevines consistently under various climatic conditions. The antagonist also survived in the phylloplane for at least seven weeks in 1996 (from the bunch closure application to véraison sampling time) and for eight weeks in 1998 (from the pea-size application to the véraison sampling time). Latorre *et al.* (1997) found that unformulated preparations of isolates S10B, P1 and Trichodex (T39) could be detected 33 days after application to flowers and 19 days after application to berries. When formulated or unformulated preparations of isolate P1 were applied this isolate could be reisolated after 21 and 28 days on berries and leaves respectively.

When *B. cinerea* and *T. harzianum* were grown together at 20°C for 14 days, *T. harzianum* covered 69% of the Petri dish and *B. cinerea* only covered 31% (Jalil *et al.,* 1997). This means, that even though 20°C is not the optimum temperature for *T. harzianum* growth, and even though this temperature is nearer to the optimum temperature for *B. cinerea* growth (23.8°C), the biocontrol agent was still able to outcompete the pathogen at this temperature. This can explain why *T. harzianum* still managed to effectively colonize the grapevine material under conditions that were not optimal for the establishment of the biocontrol agent.

It is possible to select for biocontrol agents with a lower optimal temperature and to investigate these biocntrol agents in greater detail. In bioassays conducted on dead onion leaves, *U. atrum* suppressed sporulation of *B. cinerea* and *B. aclada* by more than 85% from 6 to 24°C (Köhl *et al.,*1999). On dead cyclamen leaves, *G. roseum* was more efficient than

*U. atrum* at 21°C and 24°C, but in contrast to *U. atrum*, showed no antagonistic activity below 21°C. On dead hydrangea leaves, *U. atrum* significantly reduced sporulation of *B. cinerea* at 3°C and 1°C. Under Dutch growing conditions, the mean air temperature during leaf wetness periods in onion and lily fields is below 15°C and rarely above 20°C. In greenhouse crops the temperature is 17°C during high humidity periods. Köhl *et al.* (1999), concluded that *U. atrum* was better adapted to temperatures which occur in the field, the greenhouse crops or during cold storage than *G. roseum*.

It is not possible to comment on the effectivity of the various antagonists in the three seasons during which the trials were performed. The consistently low and sporadic occurrences of *B. cinerea* in the vineyard made this difficult. Climatic conditions are most likely responsible for this phenomenon. Temperatures were not optimal for *B. cinerea* development during the last three growing seasons. The optimal temperature for *B. cinerea* is 23°C (Jalil *et al.,* 1997). The average temperature recorded over the growing season were lower than this optimum (Figure 1). There were periods conducive to infection, but these periods did not occur frequently enough or in succession to cause disease of an epidemic level.

The observations on the occurrence of *B. cinerea* in Chardonnay bunches confirmed the findings of other workers (Holz *et al.*, 1997, 1998; Holz, 1999; Gütschow, 2001), which showed that natural latent *B. cinerea* infections generally occur in the structural parts of grape bunches, and is therefore not exclusive to the berry. The observations also confirmed that berry infections originate most frequently from pedicels. The highest levels of disease were furthermore recorded during the pea-size stage, and the lowest during véraison. Although no conclusive remarks could be made on the ability of the antagonists to control the pathogen, it is of interest to note that, during the pea-size stage in 1996, when high levels of *B. cinerea* were recorded, *T. harzianum* controlled these infections in the pedicels more effectively than any other treatment. This finding suggests that *T. harzianum* has the greatest potential to be used as a component in an integrated system.

## LITERATURE CITED

Boff, P., Geriaf, M. , Horsten, P., Lombaers-van der Plas, C.H. & Köhl, J. 1998. Sporulation suppression of *Botrytis cinerea* by *Ulocladium atrum* on strawberries. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 2: 2.10.9. (Abstract).

Cook, J. R. 1993. Making greater use of introduced micro-organisms for biological control of plant pathogens. *Annual Review of Phytopathology* 31: 53-80.

De Kock, P.J. & Holz, G. 1991. Colonisation of table grapes by *Botrytis cinerea* in the Western Cape Province. *Phytophylactica* 23: 73-80.

De Kock, P.J. & Holz, G. 1994. Application of fungicides against postharvest *Botrytis cinerea* rot of table grapes in the Western Cape. *South African Journal of Enology and Viticulture* 15: 33-40.

Dubos, B. 1992. Biological control of *Botrytis*: state-of-the-art. Pages 169-178 in: Recent advances in *Botrytis* research. K. Verhoeff, N.E. Malathrakis and B. Williamson, eds. Pudoc, Wageningen.

Elad, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. *Crop Protection* 13: 35-38.

Elad, Y., Stienberg, D. & Niv, A. 1994. *Trichoderma harzianum* T39 integrated with fungicides: improved biocontrol of grey mould. *Proceedings of the Brighton Crop Protection Conference, Pests and Diseases* 3: 1109-1114.

Faretra, F. & Pollastro, S. 1993. Isolation, characterisation and genetic analysis of laboratory mutants of *Botryotinia fuckeliana* resistant to the phenylpyrrole fungicide CGA 173506. *Mycological Research* 97: 620-624.

Ferreira, J.H.S. 1990. In vitro evaluation of epiphytic bacteria from table grapes for the suppression of *Botrytis cinerea*. *South African Journal of Enology and Viticulture* 11: 38-41.

Fourie, P.H. & Holz, G. 1998. Frequency of dicarboximide resistant strains of *Botrytis cinerea* in South African table grape vineyards and influence of spray schedules on resistant sub-populations. *South African Journal of Enology and Viticulture* 1: 3-9.

Gindrat, D. and Pezet, R. 1994. Le paraquat, un outil pour la révélation rapide d'infections fongiques latentes et de champignons endophytes. *Journal of Phytopathology* 141: 86-98.

Gütschow, M. 2001. Resistance to *Botrytis cinerea* in parts of leaves and bunches of grapevine. MSc.Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Harman, G.E., Latorre, B., Agosin, E., San Martin, R., Riegel, D.G., Nielsen, P.A., Tronsmo, A. and Pearson, R.C. 1996. Biological and integrated control of Botrytis bunch rot of grape using *Trichoderma* spp. *Biological Control* 7: 259-266.

Harvey, J.M. 1955. A method of forecasting decay in California storage grapes. *Phytopathology* 45: 229-232.

Holz, G. 1999. Behaviour and infection pathways of diverse fungal pathogens on fruit. Conference Handbook, 12th Biannual Australasian Plant Pathology Society Conference, Canberra, Australia, p 257 (Abstract).

Holz, G., Coertze, S. & Basson, E.J. 1997. Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. Annual Meeting of the American Phytopathological Society, Rochester, USA. *Phytopathology* 87: S88 (Abstract).

Holz, G., Coertze, S. & Basson, E.J. 1998. Infection pathways and latency of *Botrytis cinerea* in grape bunches. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 3: 6.22 (Abstract).

Jalil, R.C., Norero, S.A. & Apablaza, H.G. 1997. Effects of temperature on mycelial growth of *Botrytis cinerea* and its antagonist *Trichoderma harzianum*. *Ciencia-e-Investigacion-Agraria* 24: 125-132.

Janisiewicz, W. 1988. Biological control of diseases of fruits. Pages 153-165 in: Biocontrol of Plant Diseases, Volume II. Muckerji, K. G. and Garg, K. L., eds. CRC Press, Inc. Boca Raton, Florida.

Jarvis, W.R. 1980. Epidemiology. Page 219 in: The Biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore trap. *Netherlands Journal of Plant Pathology* 141: 86-98.

Köhl, J., Molhoek, W.M.L., van der Plas, C.H. & Fokkema, N.J. 1995a. Suppression of sporulation of *Botrytis* spp. as valid biocontrol strategy. *European Journal of Plant Pathology* 101: 251-259.

Köhl, J., van der Plas, C.H., Molhoek, W.M.L. & Fokkema, N.J., 1995b. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. *European Journal of Plant Pathology* 101: 627-637.

Köhl, J., Molhoek, W.M.L., van der Plas, C.H. & Fokkema, N.J. 1995c. Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed to field conditions. *Phytopathology* 85: 393-401.

Köhl, J., Bélanger, R.R. & Fokkema, N.J. 1997. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: a comparative microscopic and ultrastructural study. *Phytopathology* 87: 634-642.

Köhl, J., Gerlagh, M., De Haas, B.H. & Krijger, M.C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. *Phytopathology* 88: 568-575.

Köhl, J., van der Plas, C.H.L., Molhoek, W.M.L., Kessel, G.J.T. & van der Geijn, H.M.G. 1999. Competitive ability of the antagonists *Ulocladium atrum* and *Gliocladium roseum* at temperature favourable for *Botrytis* spp. development. *BioControl* 44: 329-346.

Latorre, B.A., Agosin, E., San Martin, R. & Vásquez, G.S. 1997. Effectiveness of conidia of *Trichoderma harzianum* produced by liquid fermentation against Botrytis bunch rot of table grape in Chile. *Crop Protection* 16: 209-214.

McClellan, W.D. & Hewitt, B. 1973. Early *Botrytis* rot of grapes: time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* 63: 1151-1157.

McLaughlin, R.J., Wisniewski, M.E., Wilson, C.L. & Chalutz, E. 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. *Phytopathology* 80: 456-461.

Nair, N.G. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. *Australian Journal of Agricultural Research* 36: 435-442.

Nair, N.G. & Hill, G.K. 1992. Bunch rot of grapes caused by *Botrytis cinerea*. Pages 147-169 in: Plant Diseases of International Importance. Vol III: Diseases of Fruit Crops. J. Kumar, H.S. Chaube, U.S. Singh and A.N. Mukhopadhyoy, eds. Prentice-Hall. New Jersey.

Nair, N.G. & Parker, F.E. 1985. Midseason bunch rot of grapes: an unusual disease phenomenon in the Hunter Valley, Australia. *Plant Pathology* 34: 302-305.

O'Neill, T.M., Elad, Y., Shtienberg, D. and Cohen, A. 1996. Control of grapevine grey mould with *Trichoderma harzianum* T39. *Biocontrol Science and Technology* 6: 139-146.

Peng, G. & Sutton, J.C. 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Canadian Journal of Plant Pathology* 13: 247-257.

Peng, G., Sutton, J.C. & Kevan, P.G. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 14: 117-129.

Pezet, R. & Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans les grappes de *Vitis vinifera* (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18: 317-322.

Savage, S.D. & Sall, M.A. 1982. The use of a radio-immunosorbent assay for *Botrytis cinerea*. *European Plant Protection Bulletin* 12: 49-53.

Smilanick, J.L. 1994. Strategies for the isolation and testing of biocontrol agents. Pages 25-41 in: Biological Control of Postharvest Diseases Theory and Practice. C. L. Wilson and M. E. Wisniewski, eds. CRC Press, Inc.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical Methods. 7th Edition. Iowa State University Press, Iowa, U.S.A.

Spurr, H.W. & Knudsen, G.R. 1985. Biological control of leaf diseases with bacteria. Pages 45-62 in: Biological Control Strategies on the Phylloplane. C.E. Windels and S. Lindow eds. APS book, St. Paul, MN.

Sutton, J.C. 1995. Evaluation of micro-organisms for biocontrol: *Botrytis cinerea* and strawberry, a case study. Pages 173-190 in: Advances in Plant Pathology. Vol. II, Sustainability and Plant Pathology. J.H. Andrews and I.C. Tommerup, eds. Academic Press, London.

Sutton, J.C. & Peng, G. 1993. Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Annual Review of Phytopathology* 31: 473-493.

Sutton, J.C., Li, D.-W., Peng, G., Yu, H., Zhang, P. & Valdebenito-Sanhueza, R.M. 1997. *Gliocladium roseum,* a versatile adversary of *Botrytis cinerea* in crops. *Plant Disease* 81: 316-328.

Williamson, L. 1997. Biocontrol of *Botrytis cinerea* on table grapes. MSc.Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Yu, H. & Sutton, J.C. 1997. Morphological development and interactions of *Gliocladium roseum* and *Botrytis cinerea* in raspberry. *Canadian Journal of Plant Pathology* 19: 237-246.

Yu, H. & Sutton, J.C. 1998. Effects of inoculum density, wetness duration, and temperature on control of *Botrytis cinerea* by *Gliocladium roseum* in raspberry. *Canadian Journal of Plant Pathology* 20: 243-252.

Zhang, P.G., Sutton, J.C. & Hopkin, A.A. 1994. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in container-grown black spruce seedlings. *Canadian Journal of Forest Research* 24: 1312-1316.

Zhang, P.G., Sutton, J.C. & Hopkin, A.A. 1996a. Inoculum concentration and time of application of *Gliocladium roseum* in relation to biocontrol of *Botrytis cinerea* in black spruce seedlings. *Canadian Journal of Forest Research* 26: 360-367.

Zhang, P.G., Sutton, J.C., Tan, W. & Hopkin, A.A. 1996b. *Gliocladium roseum* reduces physiological changes associated with infection of black spruce seedlings by *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 18: 7-13.

**Table 1.** Timing of antagonist application in the Chardonnay vineyard during the 1996/1997 and 1997/1998 seasons

| Program No. | Treatment [a] | Full bloom | Pea-size | Bunch closure | Véraison | Véraison +2wks[b] | Harvest |
|---|---|---|---|---|---|---|---|
| 1 | Control | Water | Water | Water | Water | Water | Water |
| 2 | Pyri+RB9 | Pyri | Pyri | Pyri | RB9 | RB9 | RB9 |
| 3 | Ulo+RB9 | Ulo | Ulo | Ulo | RB9 | RB9 | RB9 |
| 4 | Glio+RB9 | Glio | Glio | Glio | RB9 | RB9 | RB9 |
| 5 | Trich+RB9 | Trich | Trich | Trich | RB9 | RB9 | RB9 |
| 6 | *U. atrum* | Ulo | Ulo | Ulo | Ulo | Ulo | Ulo |
| 7 | *G. roseum* | Glio | Glio | Glio | Glio | Glio | Glio |
| 8 | *T. harzianum* | Trich | Trich | Trich | Trich | Trich | Trich |
| 9 | Pyri + Ipro | Pyri | Pyri | Pyri | Ipro | Ipro | Ipro |

[a] Pyri = Pyrimethanil; RB9 = *Trichosporon pullulans; Ulo = Ulocladium atrum; Glio = Gliocladium roseum; Trich = Trichoderma harzianum;* Ipro = Iprodione.
[b] The Véraison + 2wks spray was not applied in the1997/1998 season.

**Table 2.** Timing of antagonist application in the Chardonnay vineyard during the 1998/1999 season

| Program No. | Treatment [a] | Full bloom | Pea-size | Bunch closure | Véraison | Harvest |
|---|---|---|---|---|---|---|
| 1 | Control | Water | Water | Water | Water | Water |
| 2 | RB9 | RB9 | RB9 | RB9 | RB9 | RB9 |
| 3 | Ipro + RB9 | Ipro | Ipro | RB9 | RB9 | RB9 |
| 4 | Pyri + RB9 | Pyri | Pyri | RB9 | RB9 | RB9 |
| 5 | Trich + RB9 | Trich | Trich | RB9 | RB9 | RB9 |

[a] RB9 = *Trichosporon pullulans*; Ipro = Iprodione; Pyri = Pyrimethanil; *Trich = Trichoderma harzianum*

**Table 3.** Temperature and rainfall recorded on the day of treatment application as well as on the following day for the 1996/1997 season

| Stage | Date | Temperature (°C) | Rainfall (mm) |
|---|---|---|---|
| Full Bloom | 22-Nov | 15.27 | 0.00 |
| | 23-Nov | 16.26 | 0.00 |
| Pea-size | 11-Dec | 17.61 | 0.00 |
| | 12-Dec | 18.14 | 0.00 |
| Bunch Closure | 23-Dec | 19.03 | 0.00 |
| | 24-Dec | 18.59 | 0.00 |
| Vèraison | 04-Feb | 20.65 | 0.20 |
| | 05-Feb | 21.76 | 0.00 |
| Vèraison + 2 wk | 17-Feb | 19.66 | 0.00 |
| | 18-Feb | 20.77 | 0.00 |
| Harvest | 04-Mar | 17.19 | 0.00 |

**Table 4.** Temperature and rainfall recorded on the day of treatment application as well as on the following day for the 1997/1998 season

| Stage | Date | Temperature (°C) | Rainfall (mm) |
|---|---|---|---|
| Full Bloom | 04-Nov | 20.14 | 0.00 |
| | 05-Nov | 17.16 | 1.20 |
| Pea-size | 18-Nov | 13.56 | 0.00 |
| | 19-Nov | 15.65 | 0.00 |
| Bunch Closure | 03-Dec | 15.66 | 0.00 |
| | 04-Dec | 17.14 | 0.00 |
| Vèraison | 19-Jan | 17.67 | 0.00 |
| | 20-Jan | 17.55 | 0.00 |
| Harvest | 25-Feb | 24.25 | 0.00 |

**Table 5.** Temperature and rainfall recorded on the day of treatment application as well as on the following day for the 1998/1999 season

| Stage | Date | Temperature (°C) | Rainfall (mm) |
|---|---|---|---|
| Full Bloom | 09-Nov | 22.54 | 0.00 |
| | 10-Nov | 17.46 | 0.00 |
| Pea-size | 25-Nov | 16.75 | 0.00 |
| | 26-Nov | 18.66 | 0.00 |
| Bunch Closure | 10-Dec | 22.65 | 0.00 |
| | 11-Dec | 21.77 | 0.00 |
| Vèraison | 13-Jan | 20.87 | 0.00 |
| | 14-Jan | 18.77 | 0.00 |
| Harvest | 17-Jan | 20.29 | 0.00 |

**Table 6.** Percentage moribund and dead flower parts[w] yielding the antagonist after the full bloom application[x] in 1996 and 1997

| Program No. | Treatment [y] | Flower parts (%) yielding an antagonist[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Stamens | | Calyptra | | Embryos | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 3 | *Ulo* + RB9 | 38.75 b | 0.00 c | 22.50 b | 0.00 b | 50.00 b | 0.00 b |
| 4 | *Glio* + RB9 | 26.25 bc | 0.00 c | 5.00 b | 0.00 b | 50.00 b | 0.00 b |
| 5 | *Trich* + RB9 | 100.00 a | 18.75 a | 100.00 a | 30.00 a | 100.00 a | 50.00 a |
| 6 | *U. atrum* | 40.00 bc | 0.00 c | 22.50 cb | 0.00 b | 12.50 c | 0.00 b |
| 7 | *G. roseum* | 12.50 cd | 0.00 c | 22.50 b | 0.00 b | 50.00 b | 0.00 b |
| 8 | *T. harzianum* | 100.00 a | 10.00 b | 100.00 a | 27.50 a | 100.00 a | 25.00 ab |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 7.** Percentage moribund and dead flower parts[w] yielding the antagonist after the full bloom application[x] in 1998

| Program No. | Treatment [y] | Flower parts (%) yielding an antagonist[z] | | |
|---|---|---|---|---|
| | | Stamens | Calyptra | Embryos |
| 5 | *Trich*+ RB9 | 0.00 | 0.00 | 18.75 |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 8.** Percentage berries and rachises[w] yielding the antagonists after the full bloom application[x] in 1996 and 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | | |
|---|---|---|---|---|---|
| | | Rachises | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 |
| 3 | *Ulo* + RB9 | 80.00 abc | 10.00 bc | 76.25 b | 20.63 b |
| 4 | *Glio* + RB9 | 60.00 cd | 0.00 c | 76.25 b | 3.75 c |
| 5 | *Trich* + RB9 | 100.00 a | 40.00 a | 98.13 a | 53.75 a |
| 6 | *U. atrum* | 80.00 bc | 30.00 a | 81.25 ab | 28.13 b |
| 7 | *G. roseum* | 40.00 d | 15.00 b | 66.25 b | 10.00 c |
| 8 | *T. harzianum* | 90.00 ab | 40.00 a | 88.75 b | 58.75 a |

[w] Bunches were collected 2 wk after the full bloom application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 9.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the pea size application[x] in 1996 and 1997

| Program No. | Treatment[y] | Bunch parts (%) yielding the antagonists[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rachises | | Pedicels | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 3 | *Ulo* + RB9 | 0.00 c | 0.00 a | 0.00 d | 0.00 c | 0.00 c | 0.00 b |
| 4 | *Glio* + RB9 | 0.00 c | 1.15 a | 11.98 b | 19.95 b | 71.25 b | 4.99 a |
| 5 | *Trich* + RB9 | 13.26 a | 1.88 a | 100.00 a | 93.75 a | 100.00 a | 0.00 a |
| 6 | *U. atrum* | 0.00 c | 0.00 a | 0.00 d | 0.00 c | 0.00 c | 0.00 a |
| 7 | *G. roseum* | 0.00 c | 1.10 a | 5.46 c | 38.88 b | 57.97 b | 3.97 b |
| 8 | *T. harzianum* | 8.85 b | 0.00 a | 100.00 a | 82.60 a | 100.00 a | 0.00 a |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 10.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the pea size application[x] in 1998

| Program No. | Treatment[y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 5 | *Trich* + RB9 | 1.29 | 88.13 | 88.13 |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 11.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the bunch closure application[x] in 1996 and 1997

| Program No. | Treatment[y] | Bunch parts (%) yielding the antagonists[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rachises | | Pedicels | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 3 | *Ulo* + RB9 | 0.00 c | 0.00 bc | 0.00 d | 0.00 dc | 0.00 bc | 0.00 b |
| 4 | *Glio* + RB9 | 2.11 b | 0.66 b | 69.99 b | 8.05 b | 2.59 b | 0.00 b |
| 5 | *Trich* + RB9 | 11.88 a | 15.70 a | 100.00 a | 95.71 a | 100.00 a | 95.71 a |
| 6 | *U. atrum* | 0.00 c | 0.00 bc | 0.00 e | 0.00 d | 0.00 c | 0.00 b |
| 7 | *G. roseum* | 1.47 bc | 0.00 c | 41.72 d | 12.65 bc | 2.33 bc | 0.66 b |
| 8 | *T. harzianum* | 15.38 a | 16.21 a | 100.00 a | 90.19 a | 100.00 a | 95.19 a |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 12.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the bunch closure application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 5 | *Trich* + RB9 | 0.63 | 38.83 | 0.34 |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 13.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the véraison application[x] in 1996 and 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rachises | | Pedicels | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 3 | *Ulo* + RB9 | 0.00 c | 0.00 a | 0.00 c | 0.00 a | 0.00 b | 0.00 a |
| 4 | *Glio* + RB9 | 5.81 b | 0.00 a | 38.71 b | 0.00 a | 0.00 b | 0.00 a |
| 5 | *Trich* + RB9 | 14.23 a | 0.00 a | 91.22 a | 0.00 a | 91.22 a | 0.00 a |
| 6 | *U. atrum* | 0.00 c | 0.00 a | 0.00 c | 0.00 a | 0.00 b | 0.00 a |
| 7 | *G. roseum* | 7.55 b | 0.00 a | 46.62 b | 0.00 a | 0.00 b | 0.00 a |
| 8 | *T. harzianum* | 13.11 a | 0.00 a | 92.31 a | 0.00 a | 92.31 a | 0.00 a |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo* = *Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio* = *Gliocladium roseum; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 14.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the véraison application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 5 | *Trich* + RB9 | 0.00 | 10.05 | 0.00 |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 15.** Number of *Botrytis cinerea* infection periods recorded before sampling in the Chardonnay vineyard in the Stellenbosch area in the 1996, 1997 and 1998 seasons

| Sampling Stage | 1996 | 1997 | 1998 |
|---|---|---|---|
| Full Bloom | 3 | 0 | 5 |
| Pea size | 3 | 2 | 0 |
| Bunch Closure | 2 | 4 | 1 |
| Vèraison | 2 | 3 | 1 |

**Table 16.** Percentage moribund and dead flower parts[w] yielding *Botrytis cinerea* after the full bloom application[x] in 1996 and 1997

| Program | | Flower parts (%) yielding *B. cinerea*[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Stamens | | Calyptra | | Embryos | |
| No. | Treatment[y] | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 1 | Control | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |
| 2 | Fung + RB9 | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |
| 3 | *Ulo* + RB9 | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |
| 4 | *Glio* + RB9 | 1.25 a | 0.00 a | 2.50 a | 2.50 a | 12.50 a | 0.00 a |
| 5 | *Trich* + RB9 | 0.00 b | 0.00 a | 10.00 a | 0.00 b | 0.00 b | 0.00 a |
| 6 | *U. atrum* | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |
| 7 | *G. roseum* | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |
| 8 | *T. harzianum* | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |
| 9 | Fungicide | 0.00 b | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo* = *Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio* = *Gliocladium roseum; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 17.** Percentage moribund and dead flower parts[w] yielding *Botrytis cinerea* after the full bloom application[x] in 1998

| Program | | Flower parts (%) yielding *B. cinerea*[z] | | |
|---|---|---|---|---|
| No. | Treatment[y] | Stamens | Calyptra | Embryos |
| 1 | Control | 0.00 a | 3.75 a | 12.50 a |
| 2 | RB9 | 0.63 a | 1.25 a | 18.75 a |
| 3 | Ipro + RB9 | 0.00 a | 1.25 a | 25.00 a |
| 4 | Pyri + RB9 | 0.63 a | 2.50 a | 18.75 a |
| 5 | *Trich* + RB9 | 0.00 a | 3.75 a | 6.25 a |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 18.** Percentage berries and rachises[w] yielding *Botrytis cinerea* after the full bloom application[x] in 1996 and 1997

| Program No. | Treatment[y] | Bunch parts (%) yielding *B. cinerea*[z] | | | |
|---|---|---|---|---|---|
| | | Rachises | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 |
| 1 | Control | 5.00 a | 0.00 a | 1.25 a | 0.63 a |
| 2 | Fung + RB9 | 0.00 a | 0.00 a | 0.00 a | 1.25 a |
| 3 | *Ulo* + RB9 | 0.00 a | 0.00 a | 0.00 a | 0.63 a |
| 4 | *Glio* + RB9 | 0.00 a | 0.00 a | 3.15 a | 1.88 a |
| 5 | *Trich* + RB9 | 0.00 a | 0.00 a | 0.00 a | 0.00 a |
| 6 | *U. atrum* | 5.00 a | 0.00 a | 4.38 a | 1.25 a |
| 7 | *G. roseum* | 10.00 a | 0.00 a | 6.88 a | 1.25 a |
| 8 | *T. harzianum* | 0.00 a | 0.00 a | 1.25 a | 0.63 a |
| 9 | Fungicide | 0.00 a | 0.00 a | 0.00 a | 3.13 a |

[w] Bunches were collected 2 wk after the full bloom application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo* = *Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio* = *Gliocladium roseum; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 19.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the pea size application[x] in 1996 and 1997

| Program No. | Treatment[y] | Bunch parts (%) yielding the antagonists[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rachises | | Pedicels | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 1 | Control | 0.98 ab | 0.00 d | 67.57 a | 2.00 bc | 2.02 ab | 0.00 a |
| 2 | Fung + RB9 | 0.00 bc | 0.00 dc | 52.38 a | 1.67 bc | 3.16 a | 3.19 a |
| 3 | *Ulo* + RB9 | 3.27 a | 0.00 a | 57.71 a | 1.42 bc | 3.31 a | 1.52 a |
| 4 | *Glio* + RB9 | 0.00 bc | 0.00 d | 43.19 ab | 12.47 a | 1.75 ab | 4.76 a |
| 5 | *Trich* + RB9 | 0.00 abc | 0.00 dc | 0.00 e | 0.00 c | 0.00 bc | 1.78 a |
| 6 | *U. atrum* | 0.00 bc | 0.00 ab | 45.88 ab | 6.90 ab | 0.56 bc | 4.43 a |
| 7 | *G. roseum* | 0.00 bc | 0.00 bcd | 25.17 bc | 4.37 b | 2.27 ab | 1.98 a |
| 8 | *T. harzianum* | 0.00 c | 0.00 cd | 11.40 d | 2.37 bc | 0.00 c | 1.73 a |
| 9 | Fungicide | 0.00 bc | 0.00 d | 19.35 dc | 3.67 b | 1.02 bc | 4.11 a |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo* = *Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio* = *Gliocladium roseum; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 20.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the pea size application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 0.00 a | 2.71 ab | 0.27 ab |
| 2 | RB9 | 0.00 a | 0.45 b | 0.49 ab |
| 3 | Ipro + RB9 | 0.00 a | 1.24 ab | 0.00 b |
| 4 | Pyri + RB9 | 0.00 a | 3.12 a | 1.77 a |
| 5 | *Trich* + RB9 | 0.00 a | 0.69 ab | 0.23 b |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 21.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the bunch closure application[x] in 1996 and 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rachises | | Pedicels | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 1 | Control | 1.25 a | 0.00 b | 17.35 ab | 2.92 ab | 2.44 a | 8.40 a |
| 2 | Fung + RB9 | 0.00 b | 0.00 b | 0.00 c | 2.27 ab | 0.00 bc | 0.00 c |
| 3 | *Ulo* + RB9 | 0.00 ab | 0.78 a | 1.52 c | 0.00 b | 0.00 abc | 2.34 abc |
| 4 | *Glio* + RB9 | 0.00 b | 0.00 b | 0.00 c | 6.31 ab | 0.00 bc | 3.29 abc |
| 5 | *Trich* + RB9 | 0.00 b | 0.00 ab | 0.00 c | 0.71 ab | 0.00 c | 0.81 bc |
| 6 | *U. atrum* | 0.78 ab | 0.00 b | 20.93 a | 5.32 a | 1.28 ab | 0.00 bc |
| 7 | *G. roseum* | 0.00 b | 0.00 b | 3.75 bc | 4.77 ab | 1.25 abc | 0.63 bc |
| 8 | *T. harzianum* | 0.00 ab | 0.00 ab | 0.00 c | 1.52 ab | 0.00 abc | 0.00 bc |
| 9 | Fungicide | 0.00 b | 0.00 ab | 0.00 c | 2.40 ab | 0.00 bc | 7.07 ab |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo* = *Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio* = *Gliocladium roseum; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 22.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the bunch closure application[x] in 1998

| Program No. | Treatment[y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 1.39 a | 4.48 a | 1.66 a |
| 2 | RB9 | 0.00 b | 3.09 a | 0.59 ab |
| 3 | Ipro + RB9 | 0.60 ab | 3.62 a | 0.29 ab |
| 4 | Pyri + RB9 | 0.30 ab | 3.49 a | 0.00 b |
| 5 | *Trich* + RB9 | 0.00 b | 3.48 a | 0.61 ab |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 23.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the véraison application[x] in 1996 and 1997

| Program No. | Treatment[y] | Bunch parts (%) yielding the antagonists[z] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Rachises | | Pedicels | | Berries | |
| | | 1996 | 1997 | 1996 | 1997 | 1996 | 1997 |
| 1 | Control | 0.00 a | 0.00 a | 0.69 ab | 0.61 b | 0.00 a | 1.37 a |
| 2 | Fung + RB9 | 0.00 a | 0.00 a | 1.32 ab | 2.44 ab | 0.00 a | 3.57 a |
| 3 | *Ulo* + RB9 | 0.00 a | 0.00 a | 0.00 b | 4.21 ab | 0.00 a | 2.11 a |
| 4 | *Glio* + RB9 | 0.00 a | 0.00 a | 0.66 ab | 4.55 ab | 0.00 a | 2.29 a |
| 5 | *Trich* + RB9 | 0.00 a | 0.00 a | 0.00 b | 2.01 ab | 0.00 a | 0.78 a |
| 6 | *U. atrum* | 0.00 a | 0.00 a | 1.32 ab | 6.61 ab | 0.00 a | 1.81 a |
| 7 | *G. roseum* | 0.00 a | 0.00 a | 0.00 b | 6.34 ab | 0.00 a | 3.46 a |
| 8 | *T. harzianum* | 0.00 a | 0.00 a | 0.00 b | 8.34 a | 0.00 a | 2.92 a |
| 9 | Fungicide | 0.00 a | 0.00 a | 0.00 b | 0.00 b | 0.00 a | 0.00 a |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo* = *Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio* = *Gliocladium roseum; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 24.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the véraison application[x] in 1998

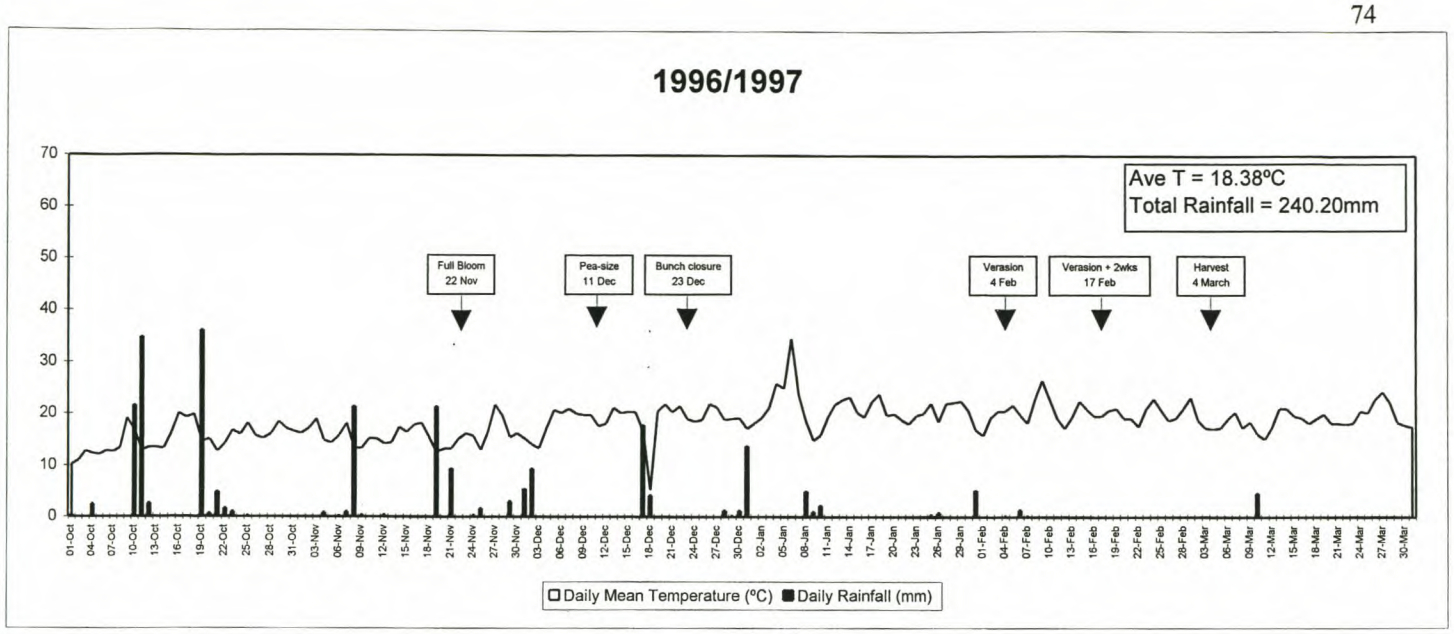
| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 0.72 a | 4.60 ab | 0.39 a |
| 2 | RB9 | 1.90 a | 7.94 a | 0.00 a |
| 3 | Ipro + RB9 | 0.00 a | 4.76 ab | 0.50 a |
| 4 | Pyri + RB9 | 0.00 a | 1.89 b | 1.07 a |
| 5 | *Trich* + RB9 | 0.45 a | 5.15 ab | 0.00 a |

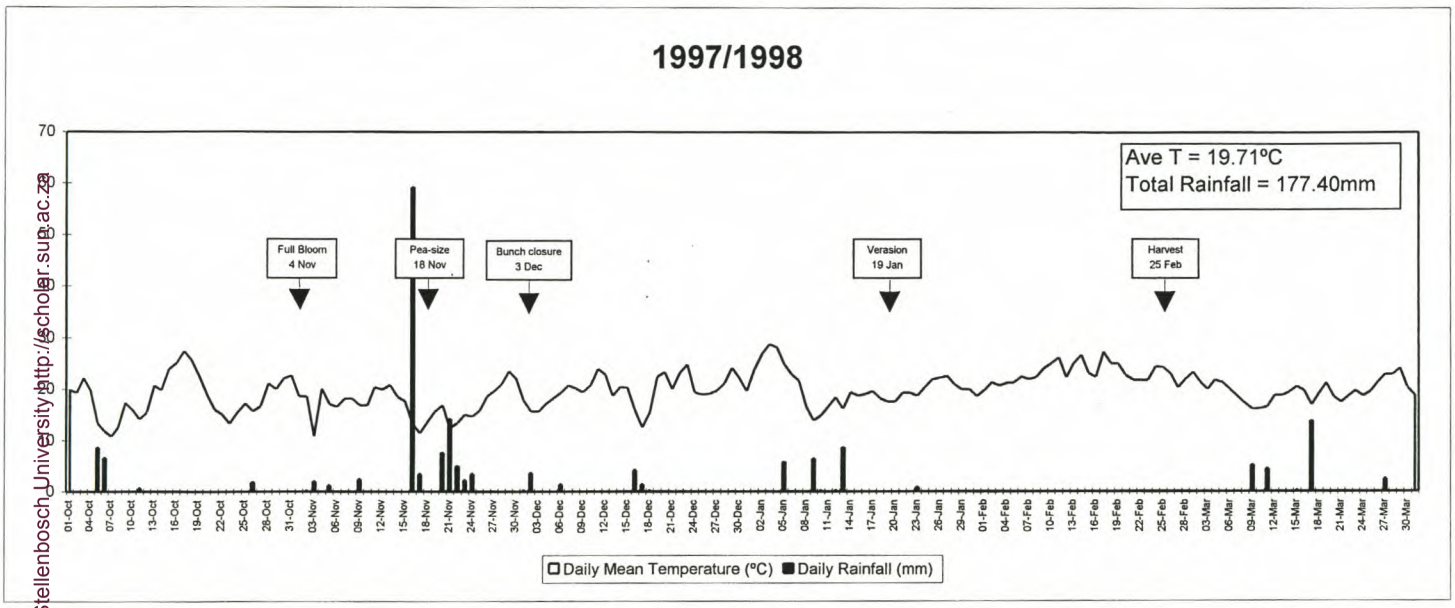
[w] Bunches were collected 2 wk after the véraison application.

[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.

[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*

[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.





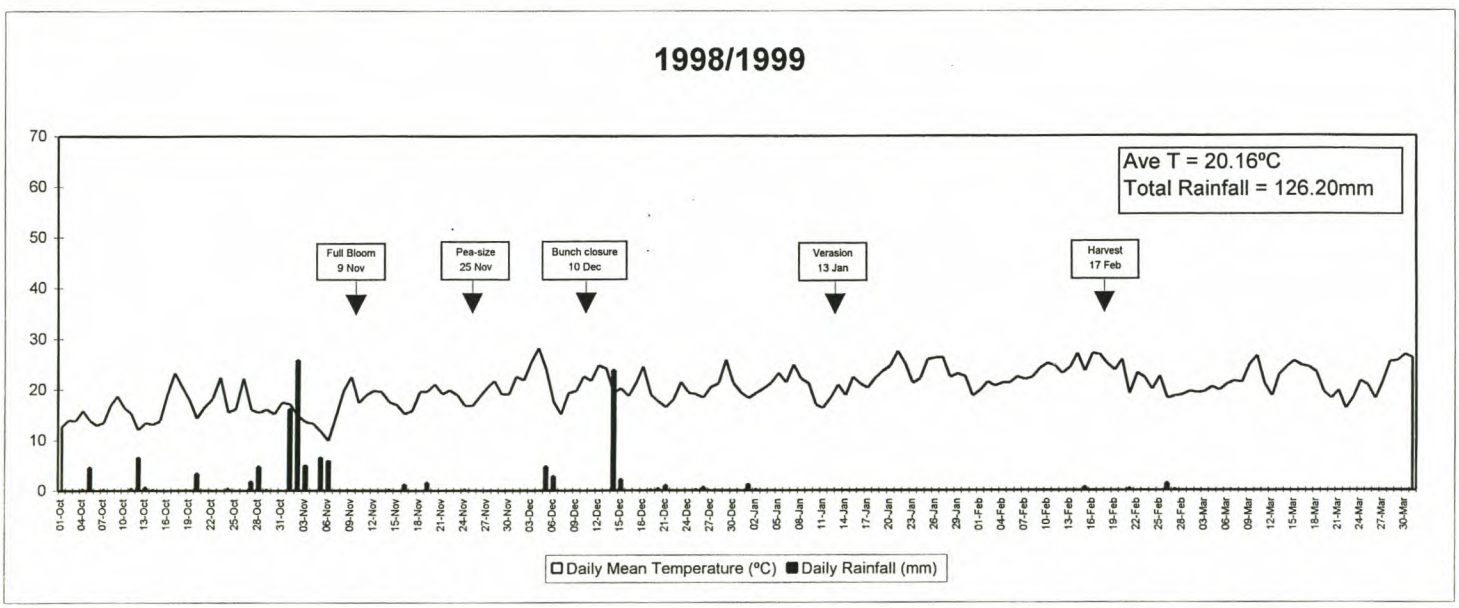




**Fig. 1.** Average daily temperature (ºC) and rainfall (mm) recorded in the Stellenbosch region in the 1996-1998 growing seasons.

# 4. COLONISATION OF SITES IN DAUPHINE TABLE GRAPE BUNCHES BY POTENTIAL BIOCONTROL ORGANISMS, AND THE OCCURRENCE OF *BOTRYTIS CINEREA*

## ABSTRACT

The potential of three fungal antagonists (*Gliocladium roseum, Ulocladium atrum* and *Trichoderma harzianum*) and one yeast (*Trichosporon pullulans*) to reduce *Botrytis cinerea* infection in grape bunches, was investigated in a commercial vineyard planted with the table grape cultivar Dauphine. Antagonists were applied as conidial suspensions to bunches at various phenological stages. Bunches were collected 2 wk after application, surface-sterilised and used for determining antagonist colonisation and *B. cinerea* infection at specific sites in the bunches. The antagonists colonised the different sites, but colonisation during the three seasons was inconsistent and sporadic. Virtually none of the floral debris recovered from bunches yielded the organisms in both seasons. *Trichoderma harzianum* colonised living tissue and developed from bunches sampled from pre-pea size to bunch closure. However, colonisation levels were generally low and never exeeded 50%. The organism furthermore showed preference for primarily colonising the pedicels and berries. In both seasons colonisation by *T. harzianum* dropped drastically at véraison, and the organism did not develop from any of the sites. *Gliocladium roseum* and *U. atrum,* on the other hand, displayed poor colonisation and developed erratically and at low levels from the different tissues at each sampling. *Botrytis cinerea* occurred sporadically during bunch development in bunches. It was therefore not possible to comment on the effectivity of the various antagonists in the two seasons during which the trials were performed. The findings however indicated that climatic conditions occurring in table grape vineyards in the Western Cape province are not well suited for the establishment of the isolates of the biocontrol agents tested in this study.

## INTRODUCTION

*Botrytis cinerea* Pers.:Fr., a pathogen of grapevine (*Vitis vinifera* L.), is associated with early-season infection (McClellan & Hewitt, 1973; Nair, 1985; Nair & Parker, 1985)

and infection of mature grapes favoured by late-season rains or prolonged periods of high relative humidity (Harvey, 1955; Jarvis, 1980). Infection of immature berries is often followed by a latent period, defined as the interval from infection to the display of macroscopic symptoms (McClellan & Hewitt, 1973). Natural latent *B. cinerea* infection is not exclusive to the grape pistil, as was proposed by McClellan and Hewitt (1973), but generally occurs in the other morpohological parts of grape bunches (Holz *et al.*, 1997, 1998; Holz, 1999; Gütschow, 2001). Grape skins provide an effective barrier to penetration by airborne conidia of the pathogen (Coertze & Holz, 1999; Coertze *et al.*, 2001; Gütschow, 2001) and berry infection is predominantly pedicel-associated (Pezet & Pont, 1986; Holz *et al.*, 1997, 1998; Holz, 1999; Gütschow, 2001). Wounds on berries are furthermore regarded as major entry sites for the pathogen (Coertze & Holz, 1999; Du Plessis, 1937; Hill *et al.*, 1981; Nair *et al.*, 1988). Due to the different sites of infection utilised by *B. cinerea* and the fact that the pathogen can remain latent in the grapevine tissue, disease management strategies should concentrate on the protection of the internal bunch parts against infection at the various phenological stages of growth.

Several attempts have been made to reduce *B. cinerea* in vineyards and in storage by means of biological control (Ferreira, 1990; McLaughlin *et al.*, 1990; Dubos, 1992; Sutton & Peng, 1993; O'Neill *et al.*, 1996; Williamson, 1997). It may be possible to obtain effective control of the pathogen by using different biological control agents each aimed at a different site in the grape bunch, protecting the bunch at the various phenological stages of growth and under different microclimatic conditions. The possibility of biological control of *B. cinerea* with *Trichoderma harzianum* has been investigated by a number of researchers (Elad, 1994; Harman *et al.*, 1996; O'Neill *et al.*, 1996; Latorre *et al.*, 1997). A biocontrol product, based on *T. harzianum* Rifai (T-39) and marketed in several countries as Trichodex 25 P (Makhteshim Chemical Works), is the first biocontrol agent for the control of *B. cinerea* on grapevine to be used commercially (Elad *et al.*, 1994). *Gliocladium roseum* was found to control *B. cinerea* on strawberries (Peng & Sutton, 1991; Peng *et al.*, 1992; Sutton, 1995; Sutton *et al.*, 1997), raspberry (Yu & Sutton, 1997; Sutton *et al.*, 1997; Yu & Sutton, 1998), black spruce seedlings (Zhang *et al.*, 1994; Zhang *et al.*, 1996a; Zhang *et al.*, 1996b; Sutton, *et al.*, 1997), and greenhouse crops (begonia, cyclamen, geranium, cucumber, pepper and tomato), (Sutton *et al.*, 1997). Because *B. cinerea* can survive endophytically (latently) on the grapevine, it should be possible to control the pathogen endophytically by means of *G. roseum* during the latent phase. The saprophyte *Ulocladium atrum* was found to compete

with *B. cinerea* on dead onion leaves (Köhl *et al.*, 1995a; Köhl *et al.*, 1995b; Köhl *et al.*, 1997), dead lily leaves (Köhl *et al.*, 1995c), necrotic strawberry stamens and dead strawberry leaves (Boff *et al.*, 1998), senescent cyclamen leaves (Köhl *et al.*, 1998) and to reduce the sporulation potential of the pathogen. *Botrytis cinerea* can colonise the dead, necrotic and aborted floral debris present in grape berry clusters early in the season and sporulate under conducive conditions to become a source of secondary inoculum in the vineyard to subsequently infect maturing grape berries later in the season. A reduction in the sporulation potential will lead to a reduction in the secondary inoculum and therefore to a reduction in decay of mature berries. The yeast *Trichosporon pullulans* was found to be effective in controlling *B. cinerea* on the grape berry surface late in the season (Williamson, 1997).

It was recently shown (Part 3) that *U. atrum*, *G. roseum*, *T. harzianum* and *T. pullulans* can each contribute to the reduction of *B. cinerea* on the wine grape Chardonnay by colonising the different niches available to the pathogen. This study reports on an investigation into the possibility of controlling *B. cinerea* on a table grape cultivar, Dauphine, by investigating the colonisation under field conditions of different sites in bunches by the three fungal antagonists and the yeast.

## MATERIALS AND METHODS

**Vineyards.** Experiments were conducted during the 1997/98-1998/99 growing seasons in experimental plots in a commercial vineyard of the table grape cultivar Dauphine in the Paarl region. Vineyard blocks ranged from 1-5 ha and the vines were trained to a slanting trellis at 3 x 1.5 m spacings. All vines were micro-irrigated. Canopy management and bunch preparation were done according to the guidelines of Van der Merwe *et al.* (1991). The normal downy and powdery mildew programs were followed, taking into account the results reported in Part 2.

**Antagonists.** Unpatented isolates of the different biocontrol agents were used in the experiments. *Gliocladium roseum* was supplied by J.C. Sutton, University of Guelph, Ontario, Canada, and *Ulocladium atrum* by J. Köhl, DLO-Research Institute for Plant Protection, Wageningen, the Netherlands. *Trichoderma harzianum* and *T. pullulans* were obtained from J.H.S. Ferreira, ARC – Fruit, Vine and Wine Research Institute, Stellenbosch. Cultures of *G. roseum* and *T. harzianum* were maintained on potato dextrose agar (PDA) at

22°C. *Ulocladium atrum* was maintained on oatmeal agar (OMA, 20 g oatmeal, 15 g agar and 1 L distilled water) at 22°C (Köhl *et al.*, 1997). *Trichosporon pullulans* was cultivated on PDA amended with chloromycetin, incubated at 30°C in the dark.

Inocula of the antagonists were prepared on different grain media. *Gliocladium roseum* was propagated on wheat grains in 1 L consol glass jars (J.C. Sutton, personal communication). Each jar contained 250 g of wheat with an equal volume of distilled water. Jars were steamed for 3 h and allowed to stand overnight to allow contaminating bacteria and yeasts to germinate before the final sterilisation process. Jars containing steamed wheat were autoclaved for 20 min at 120°C. Water remaining in the jars was poured out in a laminar flow cabinet before inoculation of the wheat. Once the wheat had cooled down each jar was inoculated with 10 ml of a spore suspension containing 1 x $10^7$ conidia/ml. The jars were kept at 22°C and the lids opened every 4-5 days to allow an exchange of air. Jars were shaken every 1-2 days and after 2 wk, the metal lids were replaced with sterilised filter paper discs to allow slow drying of the wheat, which facilitates high levels of spore production (Zhang *et al.*, 1996a). Once spore production began, the jars were exposed to black light to accelerate spore production. After 30 days the grains were covered in a mass of light pinkish, orange conidia.

*Ulocladium atrum* was propagated on oat grains in 250 ml Erlenmeyer flasks according to the method used by Köhl *et al.* (1995c). Each flask contained 30 g oats and an equal volume of distilled water. Flasks were sealed with cotton wool plugs covered in aluminum foil. The oat grains were steamed and sterilised in the same way as the wheat. Each flask was inoculated with 5 ml of a spore suspension containing 1 x$10^7$ conidia/ml. Flasks were shaken every 2-4 days and incubated at 18°C in the dark for 28 days.

*Trichoderma harzianum* was propagated on wheat grains. One liter Erlenmeyer flasks were filled with 30 g of wheat and an equal volume of distilled water. Wheat grains were sterilised by autoclaving at 120°C for 20 min, twice, consecutively. Once the grains had cooled down they were inoculated with a spore suspension containg 1 x $10^7$ conidia/ml. Flasks were incubated for 2 wk at 22°C under normal light and shaken periodically.

Fungal spore suspensions were prepared by suspending the colonised grains in sterilised, distilled water containing 0.01% Tween 80, and placed on a rotary shaker for 30 min. The

suspension was filtered through a double layer of sterile cheesecloth. Concentrations of spore suspensions were determined with a heamocytometer and adjusted to 1 x $10^6$ conidia/ml with sterile distilled water containing 0.01% Tween 80. Germination was estimated on PDA for *G. roseum* and *T. harzianum* and on OMA for *U. atrum.* Germination consistently exceeded 95% for all three antagonists.

*Trichosporon pullulans* was propagated in 1000 ml Nutrient Yeast Dextrose Broth (NYDB, 8g nutrient broth, 5g yeast extract and 10g D-glucose, suspended in one liter distilled water) in 2 L Schott bottles. The medium was inoculated with yeast cells and placed in a rotary incubator at 180 rpm for 20 h at 30°C. A yeast cell suspension was prepared by centrifuging yeast medium at 8000 rpm for 5 min at 20°C. The supernatant was poured off and the yeast cells resuspended with a 0.7% salt solution. Concentrations were estimated from standard curves for cfu/ml suspension and absorbance at 630 nm on a spectrophotometer (Ultraspec 2000 [U/V Visible Spectrophotometer] Pharmacia Biotech). Concentrations were adjusted to 1 x $10^7$ cfu/ml with a 0.7% salt solution.

**Spray Programmes.** During the 1997/98 season, the fungal antagonists were applied in combination with the yeast, and the fungicide standard iprodione (Rovral Flo 25 SC, Rhône Poulenc Agrichem) (Table 1). In the 1998/1999 season, the fungal antagonist found most effective during the first season was applied in combination with the yeast and two fungicide standards iprodione (Rovral Flo 25 SC, Rhône Poulenc Agrichem) and pyrimethanil (Scala SC 40, AgrEvo) (Table 2). Control treatments consisted of water plus 0.01% Tween 80. Treatments were applied to single-row plots, each consisting of six mature vines. Rows used in the experiment were separated from the commercial vines by untreated buffer rows. Each treatment was conducted as a completely randomised design with four replicates in the first two seasons and eight replicates in the last season. Treatments were applied with hand held equipment until runoff.

**Colonisation of bunch parts by antagonists and *B. cinerea*.** Two methods were used to determine the ability of antagonists to colonise the different sites in grape bunches, and to suppress natural infection by the pathogen. In the first method, which was conducted only during bloom, bunches were collected at the pre-pea-size stage 14 days after the application at bloom. Moribund and dead flower parts were removed from the bunches, placed on Kerssies *B. cinerea* selective medium (Kerssies, 1990) in Petri dishes and incubated at 22°C

under diurnal light. The bunches were divided in sections bearing a short section of the rachis, laterals and eight-nine berries. The sections were surface-sterilised (10 s 70% ethanol, 1 min 0.35% sodium hypochlorite, 1 s 70% ethanol) to kill the fungi on the surface, placed on paraquat-chloramphenicol agar (PQCA, 1L distilled water containing 10g Agar was autoclaved and allowed to cool, and 20 mg a.i. paraquat and 200 mg chloramphenicol were added) (Peng & Sutton, 1991) in Petri dishes and incubated at 22°C under diurnal light. Preliminary studies showed that the selective media had no effect on the antagonists. The material was regularly examined for the development of the antagonists and *B. cinerea*. The percentage sites in a bunch section yielding an antagonist or the pathogen were recorded after 14 days.

In the second method, which was conducted from pea-size stage to véraison, bunches were collected 14 days after antagonist application. The bunches were divided in sections and surface-sterilised as described previously. The sections were immersed a paraquat solution (30 ml paraquat in 1 L water for 30 sec) to terminate host resistance (Gindrat & Pezet, 1994). The sections were placed on sterile epoxy-coated steel mesh screens (53 x 28 x 2cm) in ethanol-disinfected perspex (Cape Plastics, Cape Town, South Africa) chambers (60 x 30 x 60 cm) lined with a sheet of chromatography paper with the base resting in deionised water to establish high relative humidity (≥93% RH). The chambers were kept at at 22°C under a diurnal light regime (12 h photoperiod) and the sections were regularly examined for the development of the antagonists and *B. cinerea*. The percentage sites in a bunch section yielding an antagonist or the pathogen were recorded after 14 days.

**Climatic conditions.** Temperature and rainfall for the 1997-1999 growing seasons were recorded at weather stations at Bellevue, Paarl. Periods conducive to *U. atrum* and *G. roseum* were determined according to Köhl *et al.* (1999). For both organisms optimum growth occurs at 27 – 30°C and long wetness periods. Optimum conditions for colonisation by *T. harzianum* is at 26.8°C and long wetness periods (Jalil *et al.*, 1997). Periods conducive to *B. cinerea* infection during each growing season were determined on the basis of the infection criteria of Sall *et al.* (1981). A rainy period was considered conducive to the natural development of *B. cinerea* if more than 5 mm rain was recorded during 24 h (relative humidity ≥92%; average temperature 15-22°C), or if 1-5 mm rain fell on each of two consecutive days (relative humidity ≥92%; average temperature 15-22°C).

**Statistical Analysis.** The experiments were arranged in a completely randomised design. Statistical computations were performed using the SAS (SAS Institute Inc., Cary, NC). Data was tested for normality and if necessary a suitable transformation was performed on the data. All the data was examined using analysis of variance (ANOVA) and treatment means were compared using the Student's *t*-test (Snedecor and Cochran, 1980).

## RESULTS

**Climatic conditions conducive to antagonist development.** Daily temperature and rainfall for the 1997-1999 growing seasons are shown in Fig. 1. Average temperatures for the two consecutive growing seasons were 21.43 and 20.88°C, respectively, whereas total rainfall of 93.5 and 114.6 mm rain was recorded. In both seasons rain events which might lead to humid conditions in vineyards on the day of antagonist application were not recorded at any gowth stage during the 3-day period prior to antagonist application. No rain fell during the 24 h-period following the antagonist application and prevailing temperatures were much lower than the optimum temperatures needed for maximum development by the antagonsists (Table 3-4).

**Colonisation by antagonists of moribund and dead flower parts.** In both seasons virtually none of the floral debris yielded the biocontro agents (Table 5-6).

**Colonisation by antagonists of living bunch parts.** Percentages at which the different bunch tissues yielded the antagonists during the various stages of bunch growth are given in Table 7-13. Colonisation by the antagonists are summarised below. *Trichoderma harzianum* displayed some colonisation of living tissue and developed from bunches sampled from pre-pea size to bunch closure (Table 8-11). Colonisation levels were generally low and never exeeded 50%. The organism furthermore showed preference for colonising primarily the pedicels and berries. In both seasons colonisation by *T. harzianum* dropped drastically at véraison, and the organism did not develop from any of the sites (Table 12-13). *Gliocladium roseum* and *U. atrum,* on the other hand, displayed poor colonisation and developed erratically and at low levels from the different tissues at each sampling.

**Climatic conditions conducive to *B. cinerea*.** The number of *B. cinerea* infection periods recorded before each sampling are given in Table 14. In the 1997/1998 season,

climatic conditions favoured the natural development of *B. cinerea* between the pea size and bunch closure stages. Thereafter, conditions were generally unfavourable for the development of the pathogen. In 1998/1999 season, a conducive period was recorded only prior to bloom.

**Occurrence of *B. cinerea* in moribund and dead flower parts during full bloom.** In both seasons none of the stamens and embryos yielded the pathogen (Table 15-16). The calyptra were virtually pathogen-free, except for 1997 when a small fraction yielded the pathogen in two treatments only.

**Occurrence of *B. cinerea* in living bunch parts.** Percentages at which the different bunch tissues yielded the pathogen during the various stages of bunch growth are given in Table 17-23. During the preharvest period, infection was the highest in the 1997 season in the pea size sampling (Table 18). *Botrytis cinerea* consistently developed from pedicels and berries of bunches from all treatments, but occurred at low levels in rachises. In nearly all the infected berries, the pathogen developed from the pedicel-end of the berry. The pathogen developed erratically and at low levels from the different tissues at each other preharvest sampling. The occurrence of the pathogen on cold-stored berries is given in Table 24-25. In 1997, *B. cinerea* incidences did not differ statistically between the different treatments. In 1998, *B. cinerea* incidence was significantly higher in the control than the treatment which received pyrimethanil + RB9.

**Suppression of *B. cinerea* colonisation of bunch parts.** None of the treatments with antagonists reduced the pathogen in the different sites levels significantly during the preharvest period. An exception was found in pedicels at véraison in 1998, when all treatments yielded the pathogen at significantly lower levels than the control (Table 23).

**Disease incidence on cold-stored fruit.** Although there were no statistical differences recorded between the levels of disease found on cold-stored fruit in 1997 (Table 24), there were differences in the level of disease recorded from the various treatments. The lowest level of disease was recorded on berries that had received four RB9 treatments in the season. In the 1998 season high levels of *B. cinerea* were recorded on control berries and treatment 4 (pyrimethanil and RB9) reduced the level of disease significantly (Table 25).

# DISCUSSION

A previous study (Part 3) showed that *U. atrum*, *G. roseum*, *T. harzianum* and *T. pullulans* could each contribute to the reduction of *B. cinerea* on grapevine by colonising the different niches available to the pathogen. However, it was concluded from the study which was conducted during 1996-1998 on Chardonnay wine grapes in the Stellenbosch region, that climatic conditions occurring in vineyards in the Western Cape province are not well suited for the establishment of the biocontrol agents. This study, which was conducted during 1997-1998 in vineyards of the table grape Dauphine in the Paarl region, confirmed this finding. Although the vineyards used in the two studies were only 30 km apart, the Paarl region is generally considered as warmer, and drier than Stellenbosch. In spite of the warmer climate, temperatures recorded in the vineyard on the day after antagonist application were much lower than these optimum temperatures. The optimum temperature for *U. atrum* and *G. roseum* is between 27 – 30°C (Köhl *et al.,* 1999) and for *T. harzianum* it is 26°C (Jalil *et al.,* 1997). Furthermore, no rain fell on the day before or after antagonist application. In the Chardonnay vineyard in the Stellenbosch region, *T. harzianum* colonised the grapevines consistently under various climatic conditions (Part 3). The antagonist also survived in the phylloplane for at least seven weeks in 1996 (from the bunch closure application to véraison sampling time) and for eight weeks in 1998 (from the pea-size application to the véraison sampling time). Latorre *et al.* (1997) found that unformulated preparations of isolates S10B, P1 and T39 (Trichodex) could be detected 33 days after application to flowers and 19 days after application to berries. In this study on Dauphine in the Paarl region, *T. harzianum* was not consistently found in the grape bunches, and did not occur in bunches at véraison.

It is possible to select for biocontrol agents with a lower optimal temperature and to investigate these biocntrol agents in greater detail. In bioassays conducted on dead onion leaves, *U. atrum* suppressed sporulation of *B. cinerea* and *B. aclada* by more than 85% from 6 to 24°C (Köhl *et al.,*1999). On dead cyclamen leaves, *G. roseum* was more efficient than *U. atrum* at 21°C and 24°C, but in contrast to *U. atrum*, showed no antagonistic activity below 21°C. On dead hydrangea leaves, *U. atrum* significantly reduced sporulation of *B. cinerea* at 3°C and 1°C. Under Dutch growing conditions, the mean air temperature during leaf wetness periods in onion and lily fields is below 15°C and rarely above 20°C. In greenhouse crops the temperature is 17°C during high humidity periods. Köhl *et al.* (1999),

concluded that *U. atrum* was better adapted to temperatures which occur in the field, the greenhouse crops or during cold storage than *G. roseum*.

In a previous study conducted on the wine grape Chardonnay (Part 3), no conclusive remarks could be made on the ability of the antagonists to control the pathogen. However, it was noted that, during the pea-size stage in 1996, when high levels of *B. cinerea* were recorded in Chardonnay bunches, *T. harzianum* controlled these infections in the pedicels more effectively than any other treatment. It was therefore suggested that *T. harzianum* has the greatest potential to be used as a component in an integrated system (Part 3). Similar observations on the potential of the antagonist were not made in the present study on Dauphine. This may be ascribed to the consistently low and sporadic occurrences of *B. cinerea* in the Dauphine vineyard, and climatic conditions which did not favour the antagonists.

## LITERATURE CITED

Boff, P., Geriaf, M. , Horsten, P., Lombaers-van der Plas, C.H. & Köhl, J. 1998. Sporulation suppression of *Botrytis cinerea* by *Ulocladium atrum* on strawberries. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 2: 2.10.9. (Abstract).

Coertze, S., and Holz, G. 1999. Surface colonization, penetration, and lesion formation on grapes inoculated fresh or after cold storage with single airborne conidia of *Botrytis cinerea*. *Plant Disease*. 83: 917-924.

Coertze, S., Holz, G. & Sadie, A. 2001. Germination and establishment of infection on grape berries by single airborne conidia of *Botrytis cinerea*. *Plant Disease*. 85: 668-677.

Dubos, B. 1992. Biological control of *Botrytis*: state-of-the-art. Pages 169-178 in: Recent Advances in *Botrytis* Research. K. Verhoeff, N.E. Malathrakis and B. Williamson, eds. Pudoc, Wageningen.

Du Plessis, S.J. 1937. Botrytis rot of grapes, and its control during 1933-1934. *Farming SA* 9: 395-397, 439-442.

Elad, Y. 1994. Biological control of grape grey mould by *Trichoderma harzianum*. *Crop Protection* 13: 35-38.

Elad, Y., Stienberg, D. & Niv, A. 1994. *Trichoderma harzianum* T39 integrated with fungicides: improved biocontrol of grey mould. *Proceedings of the Brighton Crop Protection Conference, Pests and Diseases* 3: 1109-1114.

Ferreira, J.H.S. 1990. In vitro evaluation of epiphytic bacteria from table grapes for the suppression of *Botrytis cinerea*. *South African Journal of Enology and Viticulture* 11: 38-41.

Gindrat, D. & Pezet, R. 1994. Le paraquat, un outil pour la révélation rapide d'infections fongiques latentes et de champignons endophytes. *Journal of Phytopathology* 141: 86-98.

Gütschow, M. 2001. Resistance to *Botrytis cinerea* in parts of leaves and bunches of grapevine. MSc.Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Harvey, J.M. 1955. A method of forecasting decay in California storage grapes. *Phytopathology* 45: 229-232.

Harman, G.E., Latorre, B., Agosin, E., San Martin, R., Riegel, D.G., Nielsen, P.A., Tronsmo, A. and Pearson, R.C. 1996. Biological and integrated control of Botrytis bunch rot of grape using *Trichoderma* spp. *Biological Control* 7: 259-266.

Hill, G.K., Stellwaag-Kittler, F., Huth, G., and Schlösser, E. 1981. Resistance of grapes in different development stages to *Botrytis cinerea*. *Phytopathologische Zeitschrift* 102: 329-338.

Holz, G. 1999. Behaviour and infection pathways of diverse fungal pathogens on fruit. Conference Handbook, 12th Biannual Australasian Plant Pathology Society Conference, Canberra, Australia, p 259. (Abstract).

Holz, G., Coertze, S. & Basson, E.J. 1997. Latent infection of *Botrytis cinerea* in grape pedicels leads to postharvest decay. Annual Meeting of the American Phytopathological Society, Rochester, USA. *Phytopathology* 87: S88. (Abstract).

Holz, G., Coertze, S. & Basson, E.J. 1998. Infection pathways and latency of *Botrytis cinerea* in grape bunches. 7th International Congress of Plant Pathology, Edinburgh, Scotland, Volume 3:6.22. (Abstract).

Jarvis, W.R. 1980. Epidemiology. Page 219 in: The Biology of *Botrytis*. J.R. Coley-Smith, K. Verhoeff and W.R. Jarvis, eds. Academic Press, London.

Jalil, R.C., Norero, S.A. & Apablaza, H.G. 1997. Effects of temperature on mycelial growth of *Botrytis cinerea* and its antagonist *Trichoderma harzianum*. *Ciencia-e-Investigacion-Agraria* 24: 125-132.

Kerssies, A. 1990. A selective medium for *Botrytis cinerea* to be used in a spore trap. *Netherlands Journal of Plant Pathology* 141: 86-98.

Köhl, J., Molhoek, W.M.L., van der Plas, C.H. & Fokkema, N.J. 1995a. Suppression of sporulation of *Botrytis* spp. as valid biocontrol strategy. *European Journal of Plant Pathology* 101: 251-259.

Köhl, J., van der Plas, C.H., Molhoek, W.M.L. & Fokkema, N.J. 1995b. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. *European Journal of Plant Pathology* 101: 627-637.

Köhl, J., Molhoek, W.M.L., van der Plas, C.H. & Fokkema, N.J. 1995c. Effect of *Ulocladium atrum* and other antagonists on sporulation of *Botrytis cinerea* on dead lily leaves exposed o field conditions. *Phytopathology* 85: 393-401.

Köhl, J., Bélanger, R.R. & Fokkema, N.J. 1997. Interaction of four antagonistic fungi with *Botrytis aclada* in dead onion leaves: a comparative microscopic and ultrastructural study. *Phytopathology* 87: 634-642.

Köhl, J., Gerlagh, M., De Haas, B.H. & Krijger, M.C. 1998. Biological control of *Botrytis cinerea* in cyclamen with *Ulocladium atrum* and *Gliocladium roseum* under commercial growing conditions. *Phytopathology* 88: 568-575.

Köhl, J., van der Plas, C.H.L., Molhoek, W.M.L., Kessel, G.J.T. & van der Geijn, H.M.G. 1999. Competitive ability of the antagonists *Ulocladium atrum* and *Gliocladium*

*roseum* at temperature favourable for *Botrytis* spp. development. *BioControl* 44: 329-346.

Latorre, B.A., Agosin, E., San Martin, R. & Vásquez, G.S. 1997. Effectiveness of conidia of *Trichoderma harzianum* produced by liquid fermentation against Botrytis bunch rot of table grape in Chile. *Crop Protection* 16: 209-214.

McClellan, W.D. & Hewitt, B. 1973. Early *Botrytis* rot of grapes: Time of infection and latency of *Botrytis cinerea* Pers. in *Vitis vinifera* L. *Phytopathology* 63: 1151-1157.

McLaughlin, R.J., Wisniewski, M.E., Wilson, C.L. & Chalutz, E. 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. *Phytopathology* 80: 456-461.

Nair, N.G. 1985. Fungi associated with bunch rot of grapes in the Hunter Valley. *Australian Journal of Agricultural Research* 36: 435-442.

Nair, N.G. & Parker, F.E. 1985. Midseason bunch rot of grapes: an unusual disease phenomenon in the Hunter Valley, Australia. *Plant Pathology* 34: 302-305.

Nair, N.G., Emmett, R.W. & Parker, F.E. 1988. Some factors predisposing grape berries to infection by *Botrytis cinerea*. *New Zealand Journal of Experimental Agriculture* 16: 257-263.

O'Neill, T.M., Elad, Y., Shtienberg, D. & Cohen, A. 1996. Control of grapevine grey mould with *Trichoderma harzianum* T39. *Biocontrol Science and Technology* 6: 139-146.

Peng, G. & Sutton, J.C. 1991. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in strawberry. *Canadian Journal of Plant Pathology* 13: 247-257.

Peng, G., Sutton, J.C. & Kevan, P.G. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 14: 117-129.

Pezet, R. & Pont, V. 1986. Infection florale et latence de *Botrytis cinerea* dans les grappes de *Vitis vinifera* (var. Gamay). *Revue Suisse de Viticulture, Arboriculture et Horticulture* 18: 317-322.

Snedecor, G.W. & Cochran, W.G. 1980. Statistical Methods. 7th Edition. Iowa State University Press, Iowa, U.S.A.

Sutton, J.C. 1995. Evaluation of micro-organisms for biocontrol: *Botrytis cinerea* and strawberry, a case study. Pages 173-190 in: Advances in Plant Pathology. Vol. II, Sustainability and Plant Pathology. J.H. Andrews and I.C. Tommerup, eds. Academic Press, London.

Sutton, J.C., Li, D.-W., Peng, G., Yu, H., Zhang, P. & Valdebenito-Sanhueza, R.M. 1997. *Gliocladium roseum,* a versatile adversary of *Botrytis cinerea* in crops. *Plant Disease* 81: 316-328.

Sutton, J.C. & Peng, G. 1993. Manipulation and vectoring of biocontrol organisms to manage foliage and fruit diseases in cropping systems. *Annual Review of Phytopathology* 31: 473-493.

Van der Merwe, G.G., Geldenhuys, P.D. & Botes, W.S. 1991. Guidelines for the preparation of table grape cultivars for export. Unifruco, Bellville.

Williamson, L. 1997. Biocontrol of *Botrytis cinerea* on table grapes. MSc.Agric. thesis, University of Stellenbosch, Stellenbosch, South Africa.

Yu, H. & Sutton, J.C. 1997. Morphological development and interactions of *Gliocladium roseum* and *Botrytis cinerea* in raspberry. *Canadian Journal of Plant Pathology* 19: 237-246.

Yu, H. & Sutton, J.C. 1998. Effects of inoculum density, wetness duration, and temperature on control of *Botrytis cinerea* by *Gliocladium roseum* in raspberry. *Canadian Journal of Plant Pathology* 20: 243-252.

Zhang, P.G., Sutton, J.C. & Hopkin, A.A. 1994. Evaluation of microorganisms for biocontrol of *Botrytis cinerea* in container-grown black spruce seedlings. *Canadian Journal of Forest Research* 24: 1312-1316.

Zhang, P.G., Sutton, J.C., Tan, W. & Hopkin, A.A. 1996a. *Gliocladium roseum* reduces physiological changes associated with infection of black spruce seedlings by *Botrytis cinerea*. *Canadian Journal of Plant Pathology* 18: 7-13.

Zhang, P.G., Sutton, J.C. & Hopkin, A.A. 1996b. Inoculum concentration and time of application of *Gliocladium roseum* in relation to biocontrol of *Botrytis cinerea* in black spruce seedlings. *Canadian Journal of Forest Research* 26: 360-367.

**Table 1.** Timing of antagonist application in the Dauphine vineyard during the 1997/1998 season

| Program No. | Treatment [a] | Full bloom | Pea-size | Bunch closure | Véraison | Harvest |
|---|---|---|---|---|---|---|
| 1 | Control | Water | Water | Water | Water | Water |
| 2 | Pyri + Ipro | Pyrimethanil | Pyrimethanil | Iprodione | | Iprodione |
| 3 | RB9 | RB9 | RB9 | RB9 | | RB9 |
| 4 | Ipro | | | Iprodione | | Iprodione |
| 5 | RB9 | | | RB9 | | RB9 |
| 6 | Pyri+RB9 | Pyrimethanil | Pyrimethanil | RB9 | RB9 | RB9 |
| 7 | Glio+RB9 | *G. roseum* | *G. roseum* | RB9 | RB9 | RB9 |
| 8 | Ulo+RB9 | *U. atrum* | *U. atrum* | RB9 | RB9 | RB9 |
| 9 | Trich+RB9 | *T. harzianum* | *T. harzianum* | RB9 | RB9 | RB9 |

[a] Pyri = Pyrimethanil; Ipro = Iprodione; RB9 = *Trichosporon pullulans;* Glio = *Gliocladium roseum;* Ulo = *Ulocladium atrum;* Trich = *Trichoderma harzianum.*

**Table 2.** Timing of antagonist application in the Dauphine vineyard during the 1998/1999 season

| Program No. | Treatment [a] | Full bloom | Pea-size | Bunch closure | Véraison | Harvest |
|---|---|---|---|---|---|---|
| 1 | Control | Water | Water | Water | Water | Water |
| 2 | RB9 | RB9 | RB9 | RB9 | RB9 | RB9 |
| 3 | Ipro + RB9 | Iprodione | Iprodione | RB9 | RB9 | RB9 |
| 4 | Pyri + RB9 | Pyrimethanil | Pyrimethanil | RB9 | RB9 | RB9 |
| 5 | Trich + RB9 | *T. harzianum* | *T. harzianum* | RB9 | RB9 | RB9 |

[a] RB9 = *Trichosporon pullulans*; Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*

**Table 3.** Temperature and rainfall recorded on the day of treatment application as well as on the following day for the 1997/1998 season

| Stage | Date | Temperature (°C) | Rainfall (mm) |
|---|---|---|---|
| Full Bloom | 30-Oct | 21.00 | 0.00 |
| | 31-Oct | 25.50 | 0.00 |
| Pea-size | 13 Nov | 19.90 | 0.00 |
| | 14-Nov | 18.90 | 0.00 |
| Bunch Closure | 9-Dec | 21.00 | 0.00 |
| | 10-Dec | 21.50 | 0.00 |
| Vèraison | 11-Feb | 28.80 | 0.00 |
| | 12-Feb | 22.80 | 0.00 |
| Harvest | 25-Feb | 31.30 | 0.00 |

**Table 4.** Temperature and rainfall recorded on the day of treatment application as well as on the following day for the 1998/1999 season

| Stage | Date | Temperature (°C) | Rainfall (mm) |
|---|---|---|---|
| Full Bloom | 09-Nov | 27.00 | 0.00 |
| | 10-Nov | 18.40 | 0.00 |
| Pea-size | 25-Nov | 18.60 | 0.00 |
| | 26-Nov | 19.80 | 0.00 |
| Bunch Closure | 10-Dec | 24.30 | 0.00 |
| | 11-Dec | 25.80 | 0.00 |
| Vèraison | 14-Jan | 19.30 | 0.00 |
| | 15-Jan | 24.30 | 0.00 |
| Harvest | 08-Mar | 21.60 | 0.00 |

**Table 5.** Percentage moribund and dead flower parts[w] yielding the antagonist after the full bloom application[x] in 1997

| Program No. | Treatment [y] | Flower parts (%) yielding an antagonist[z] | | |
|---|---|---|---|---|
| | | Stamens | Calyptra | Embryos |
| 7 | Glio + RB9 | 0.00 a | 0.00 a | 0.00 a |
| 8 | Ulo + RB9 | 0.00 a | 3.33 a | 0.00 a |
| 9 | Trich + RB9 | 0.00 a | 3.33 a | 0.00 a |

[w] Flower parts were collected 2 wk after the full bloom application.

[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.

[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*

[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 6.** Percentage moribund and dead flower parts[w] yielding the antagonist after the full bloom application[x] in 1998

| Program No. | Treatment [y] | Flower parts (%) yielding an antagonist[z] | | |
|---|---|---|---|---|
| | | Stamens | Calyptra | Embryos |
| 5 | *Trich* + RB9 | 0.00 | 2.50 | 0.00 |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 7.** Percentage berries and rachises[w] yielding the antagonists after the full bloom application[x] 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | |
|---|---|---|---|
| | | Rachises | Berries |
| 7 | Glio + RB9 | 6.67 a | 22.50 b |
| 8 | Ulo + RB9 | 0.00 a | 33.33 ab |
| 9 | Trich + RB9 | 13.33 a | 49.17 a |

[w] Bunches were collected 2 wk after the full bloom application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 8.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the pea size application[x] in 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 7 | Glio + RB9 | 3.06 ab | 13.23 ab | 10.16 ab |
| 8 | Ulo + RB9 | 0.00 b | 3.33 b | 0.00 b |
| 9 | Trich + RB9 | 5.60 a | 49.17 a | 14.22 a |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 9.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the pea size application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 5 | *Trich* + RB9 | 3.24 | 15.78 | 13.38 |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 10.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the bunch closure application[x] in 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 7 | Glio + RB9 | 5.56 ab | 24.07 ab | 0.00 a |
| 8 | Ulo + RB9 | 0.00 b | 0.00 b | 0.00 a |
| 9 | Trich + RB9 | 14.42 a | 65.88 a | 2.38 a |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 11.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the bunch closure application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 5 | *Trich* + RB9 | 8.06 | 42.98 | 40.18 |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 12.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the véraison application[x] in 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 7 | Glio + RB9 | 0.00 a | 0.00 a | 0.00 a |
| 8 | Ulo + RB9 | 0.00 a | 0.00 a | 0.00 a |
| 9 | Trich + RB9 | 0.00 a | 0.00 a | 0.00 a |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 13.** Percentage berries, pedicels and rachises[w] yielding the antagonists after the véraison application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 5 | *Trich* + RB9 | 0.00 | 0.00 | 0.00 |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages.

**Table 14.** Number of *Botrytis cinerea* infection periods recorded before sampling in the Dauphine vineyard in the Paarl area in the 1997 and 1998 seasons

| Sampling Stage | 1997 | 1998 |
|---|---|---|
| Full Bloom | 0 | 3 |
| Pea size | 0 | 0 |
| Bunch Closure | 0 | 0 |
| Vèraison | 0 | 0 |

**Table 15.** Percentage moribund and dead flower parts[w] yielding *Botrytis cinerea* after the full bloom application[x] in 1997

| Program No. | Treatment [y] | Flower parts (%) yielding *B. cinerea*[z] | | |
|---|---|---|---|---|
| | | Stamens | Calyptra | Embryos |
| 1 | Control | 0.00 a | 0.00 a | 0.00 a |
| 2 | Fungicide | 0.00 a | 0.00 a | 0.00 a |
| 3 | RB9 | 0.00 a | 0.00 a | 0.00 a |
| 4 | Fungicide | 0.00 a | 0.00 a | 0.00 a |
| 5 | RB9 | 0.00 a | 3.33 a | 0.00 a |
| 6 | Fung+RB9 | 0.00 a | 0.00 a | 0.00 a |
| 7 | Glio+RB9 | 0.00 a | 0.00 a | 0.00 a |
| 8 | Ulo+RB9 | 0.00 a | 0.00 a | 0.00 a |
| 9 | Trich+RB9 | 0.00 a | 3.33 a | 0.00 a |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 16.** Percentage moribund and dead flower parts[w] yielding *Botrytis cinerea* after the full bloom application[x] in 1998

| Program No. | Treatment [y] | Flower parts (%) yielding *B. cinerea*[z] | | |
|---|---|---|---|---|
| | | Stamens | Calyptra | Embryos |
| 1 | Control | 0.00 a | 0.00 a | 0.00 a |
| 2 | RB9 | 0.00 a | 0.00 a | 0.00 a |
| 3 | Ipro + RB9 | 0.00 a | 0.00 a | 0.00 a |
| 4 | Pyri + RB9 | 0.00 a | 0.00 a | 0.00 a |
| 5 | *Trich* + RB9 | 0.00 a | 0.00 a | 0.00 a |

[w] Flower parts were collected 2 wk after the full bloom application.
[x] Surface-sterilised flower parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 17.** Percentage berries and rachises[w] yielding *Botrytis cinerea* after the full bloom application[x] in 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding *B. cinerea*[z] | |
|---|---|---|---|
| | | Rachises | Berries |
| 1 | Control | 0.00 a | 0.83 a |
| 2 | Fungicide | 0.00 a | 4.17 a |
| 3 | RB9 | 0.00 a | 2.50 a |
| 4 | Fungicide | 0.00 a | 1.67 a |
| 5 | RB9 | 0.00 a | 2.50 a |
| 6 | Fung+RB9 | 0.00 a | 0.83 a |
| 7 | Glio+RB9 | 0.00 a | 0.83 a |
| 8 | Ulo+RB9 | 0.00 a | 2.50 a |
| 9 | Trich+RB9 | 0.00 a | 0.00 a |

[w] Bunches were collected 2 wk after the full bloom application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 18.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the pea size application[x] in 1996 and 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding *B. cinerea*[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 0.98 ab | 7.89 ab | 12.68 abc |
| 2 | Fungicide | 0.00 b | 2.57 b | 4.84 dc |
| 3 | RB9 | 1.04 ab | 5.31 ab | 3.23 d |
| 4 | Fungicide | 1.01 a | 7.23 ab | 7.82 bc |
| 5 | RB9 | 0.00 ab | 20.91 a | 23.84 a |
| 6 | Fung+RB9 | 0.00 ab | 10.41 ab | 13.67 abc |
| 7 | Glio+RB9 | 0.00 ab | 8.98 ab | 16.81 ab |
| 8 | Ulo+RB9 | 0.00 ab | 18.18 a | 8.54 bc |
| 9 | Trich+RB9 | 0.00 ab | 12.55 ab | 8.57 bcd |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 19.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the pea size application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 1.00 a | 0.00 a | 0.00 a |
| 2 | RB9 | 0.00 ab | 0.00 a | 0.78 a |
| 3 | Ipro + RB9 | 0.00 ab | 1.56 a | 0.78 a |
| 4 | Pyri + RB9 | 0.00 b | 3.35 a | 0.81 a |
| 5 | *Trich* + RB9 | 0.00 b | 0.00 a | 0.00 a |

[w] Bunches were collected 2 wk after the pea size application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 20.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the bunch closure application[x] in 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 0.00 b | 0.00 a | 0.00 b |
| 2 | Fungicide | 0.00 ab | 5.79 a | 0.00 b |
| 3 | RB9 | 0.00 b | 0.00 a | 0.00 b |
| 4 | Fungicide | 0.00 ab | 0.00 a | 0.00 ab |
| 5 | RB9 | 0.00 b | 0.00 a | 0.00 b |
| 6 | Fung+RB9 | 1.59 a | 14.29 a | 4.06 a |
| 7 | Glio+RB9 | 0.00 ab | 0.00 a | 3.70 ab |
| 8 | Ulo+RB9 | 0.00 ab | 5.13 a | 1.67 ab |
| 9 | Trich+RB9 | 0.00 ab | 0.00 a | 0.00 b |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 21.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the bunch closure application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 0.00 b | 0.00 a | 0.00 a |
| 2 | RB9 | 0.00 ab | 0.93 a | 1.04 a |
| 3 | Ipro + RB9 | 0.00 b | 0.00 a | 0.00 a |
| 4 | Pyri + RB9 | 0.00 ab | 1.14 a | 0.00 a |
| 5 | *Trich* + RB9 | 0.00 a | 1.14 a | 0.00 a |

[w] Bunches were collected 2 wk after the bunch closure application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 22.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the véraison application[x] in 1997

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 0.00 cd | 0.00 bc | 0.00 b |
| 2 | Fungicide | 0.00 d | 0.00 c | 0.00 b |
| 3 | RB9 | 0.00 abc | 3.70 abc | 1.67 ab |
| 4 | Fungicide | 0.00 bcd | 0.00 bc | 0.00 b |
| 5 | RB9 | 0.00 abc | 5.66 ab | 0.00 ab |
| 6 | Fung+RB9 | 0.00 abc | 0.00 abc | 0.00 ab |
| 7 | Glio+RB9 | 0.00 ab | 0.00 abc | 0.00 ab |
| 8 | Ulo+RB9 | 0.00 abc | 2.08 abc | 0.00 ab |
| 9 | Trich+RB9 | 0.00 a | 5.77 a | 3.70 a |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 23.** Percentage berries, pedicels and rachises[w] yielding *Botrytis cinerea* after the véraison application[x] in 1998

| Program No. | Treatment [y] | Bunch parts (%) yielding the antagonists[z] | | |
|---|---|---|---|---|
| | | Rachises | Pedicels | Berries |
| 1 | Control | 2.95 a | 11.28 a | 0.00 b |
| 2 | RB9 | 2.63 a | 2.78 b | 1.32 ab |
| 3 | Ipro + RB9 | 1.04 a | 5.37 ab | 3.13 ab |
| 4 | Pyri + RB9 | 0.00 a | 1.39 b | 6.67 a |
| 5 | Trich + RB9 | 0.00 a | 2.78 b | 0.00 b |

[w] Bunches were collected 2 wk after the véraison application.
[x] Surface-sterilised bunch parts were incubated on *B. cinerea* selective Kerssies medium for 2 wk at 22°C.
[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 24.** Percentage berries[w] yielding *Botrytis cinerea* after the harvest application[x] in 1997

| Program No. | Treatment [y] | Incidence (%)[z] |
|---|---|---|
| 1 | Control | 7.24 a |
| 2 | Fungicide | 8.38 a |
| 3 | RB9 | 3.27 a |
| 4 | Fungicide | 10.94 a |
| 5 | RB9 | 5.66 a |
| 6 | Fung+RB9 | 7.54 a |
| 7 | Glio+RB9 | 12.67 a |
| 8 | Ulo+RB9 | 9.04 a |
| 9 | Trich+RB9 | 8.17 a |

[w] Bunches were collected 2 days after the harvest application.
[x] Bunches were packed in boxes as for export and kept at 0.5°C for 2 weeks and room temperature for 1 week before visual inspection for *B. cinerea.*
[y] *Ulo = Ulocladium atrum;* RB9 = *Trichosporon pullulans; Glio = Gliocladium roseum; Trich = Trichoderma harzianum.*
[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

**Table 25.** Percentage berries[w] yielding *Botrytis cinerea* after the harvest application[x] in 1998

| Program No. | Treatment [y] | Incidence (%)[z] |
|---|---|---|
| 1 | Control | 19.57 a |
| 2 | RB9 | 9.03 ab |
| 3 | Ipro + RB9 | 8.14 ab |
| 4 | Pyri + RB9 | 7.63 b |
| 5 | Trich + RB9 | 8.21 ab |

[w] Bunches were collected 2 days after the harvest application.

[x] Bunches were packed in boxes as for export and kept at 0.5°C for 2 weeks and room temperature for 1 week before visual inspection for *B. cinerea.*

[y] RB9 = *Trichosporon pullulans;* Ipro = Iprodione; Pyri = Pyrimethanil; *Trich* = *Trichoderma harzianum.*

[z] Means in the table represent actual percentages. LSD were calculated on the logit transformed data at a significance level of 5%. Means within a column followed by a common letter do not differ significantly at $P = 0.05$ according to an LSD test.

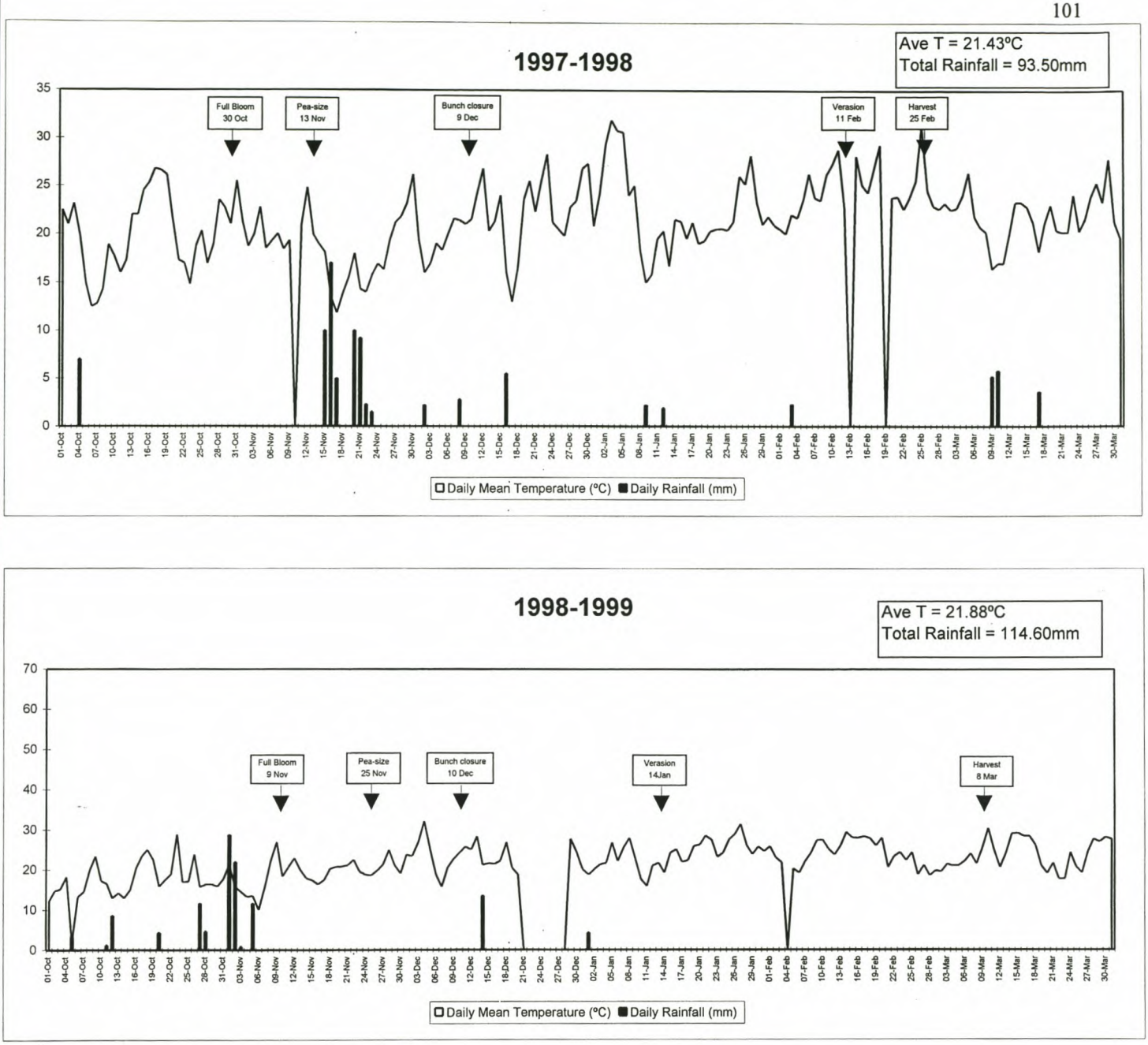



**Fig. 1.** Average daily temperature (°C) and rainfall (mm) recorded in the Paarl region in the 1997-1998 growing seasons.